



**COLD-ADAPTED INFLUENZA VIRUS  
SPONSORSHIP**

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Sequence  
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08/082846

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**BIOLOGICAL DEPOSITS**

The following viral strains have been deposited under the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852:

VIRUS	Q ACCESSION NO.	DATE OF DEPOSIT
Wild type A/Ann Arbor/6/60 (H2N2) egg passage 2(3)		June 10, 1993
Cold-adapted "Master Strain" A/Ann Arbor/6/60 7P1 (H2N2)		June 10, 1993

**FIELD OF THE INVENTION**

The present invention relates generally to cold-adapted influenza virus and, more particularly, to a cold-adapted influenza virus vaccine and methods of preventing and treating influenza by employing the vaccine.

**BACKGROUND OF THE INVENTION**

The tremendous impact of influenza virus infections on the public health is widely recognized. Control of influenza has relied primarily on the use of inactivated influenza vaccines. More current approaches, however, have moved towards the use of live attenuated vaccine. Kilbourne, E.D. "Influenza" (Plenum Publishing Corp. New York), p. 291-332 (1987). The most promising efforts in the development of an effective live vaccine have centered on adapting the virus to grow at suboptimal temperatures. Maassab, H.F., et al., *Vaccine* 3:355-369 (1985). Using this approach, cold-adapted attenuated influenza viruses have been developed in both the former Soviet Union and the United States. Alexandrova, G.I., et al., *Rev. Roum. Inframicrobil.* 2:179-189 (1965); Maassab, H.F. *Nature (London)* 213: 612-614 (1967).



In particular, cold adaptation (ca) has permitted the A/Ann Arbor/6/60 (H2N2) (A/AA/6/60) virus of the present invention to grow as well at 25°C as it does at 33°C. Maassab, H.F. *Nature (London)* 213:612-614 (1967); Maassab, H.F. "Biology of Large RNA Viruses" (Academic Press, New York), p. 542-565 (1970). The ca A/AA/6/60 virus  
5 is also temperature-sensitive (ts), a property that impedes replication at higher temperatures in the lungs and thus is highly desirable for live vaccines. Maassab, H.F., "Biology of Large RNA Viruses" (Academic Press, New York), p. 542-565 (1970); Mulder, J., et al., "Influenza" (Wolters-Noordhoff, Amsterdam), 1-6:78-80 (1972). Single-gene studies of this cold-adapted virus in a background of A/Korea/1/82 (H3N2) have  
10 identified the genes responsible for the ca and ts phenotypes and for attenuation in that gene constellation. Snyder, M.H., et al., *J. Virol.* 62(2):488-495 (1988).

Live attenuated vaccines are produced by reassorting the six internal genes of the cold-adapted A/Ann Arbor/6/60 influenza virus with the two surface genes of the currently circulating wild type (wt) virus, thereby producing a reassortant strain.  
15 Maassab, H.F. "Negative Strand Viruses" (Academic Press, New York), p. 755-763 (1975); Davenport, F.M., et al., *J. Infect. Dis.* 136:17-25 (1977). Vaccines prepared from ca A/AA/6/60 have proven both non-reactogenic and non-transmissible in preliminary field trials at six different medical centers involving over 20,000 people. Couch, R.B., et al., "Options for the Control of Influenza" (Alan R. Liss, New York), p.  
20 223-241 (1986); Wright, P.F., et al., "Options for the Control of Influenza" (Alan R. Liss, New York), p. 243-253 (1986). These vaccines also provide higher IgA levels than the killed vaccines and afford longer-lasting protection in children. Murphy, B.R., et al., *Infect. Immun.* 36(3):1102-1108 (1982); Johnson, P.R., et al., *J. Infect. Dis.* 154(1):121-127 (1986). Currently, the ca A/AA/6/60 7PI (plaque-purified seven times) master strain  
25 preparation is under development for use as a live vaccine in children and other live virus vaccines are being developed using the live ca influenza vaccine as a model.

Cold-adapted reassortant vaccines have thus been shown to have the proper level of attenuation, immunogenicity, and non-transmissibility combined with proven genetic stability and are produced in acceptable tissue culture substrates. In general,  
30 live cold-adapted reassortant vaccines offer several advantages over the existing inactivated vaccine. These include the possible use of a single dose, and administration by the natural route of infection, i.e. intranasally. In addition, ca vaccines stimulate a wide range of antibody responses, and result in induction of both local and humoral immunity. Furthermore, these vaccines are cost-effective and can  
35 be rapidly produced and updated in the event of antigenic changes. In addition, laboratory guidelines are available for the assessment of virulence (reactogenicity in



ferrets) and attenuation can be reproducibly achieved. Moreover, the presence of two phenotypic markers (the temperature-sensitive and cold-adapted phenotypes) allows for the evaluation of virulence and monitoring of the vaccine in the field.

However, despite the above-described advantages, until now virtually nothing  
5 has been known about the molecular basis of cold adaptation. Published information indicates that cold adaptation has produced one or more mutations in each of the genes encoding the internal proteins of the A/AA/6/60 master strain. Cox, N.J., et al., "Genetic Variation Among Influenza Viruses" (Academic Press), p. 639-652 (1981). However, all of the work has been done on viruses passaged 28 to 32 times in eggs  
10 in parallel with the virus passaged in primary chick kidney cells during cold adaptation. Cox, N.J., et al., *Viol.* 167:554-567 (1988). Studies, however, have shown a gradual buildup of mutations in the RNA1 of sequential 35°C egg passages 2 through 28 of wild type viruses, and recent findings have shown the influence of host cell variation on influenza viruses passaged in chicken eggs. Katz, J.M., et al., *Viol.*  
15 156:386-395 (1987). Thus, the mutations leading to cold adaptation and attenuation have heretofore been unknown.

It would thus be desirable to isolate and provide the wild type A/Ann Arbor/6/60 progenitor virus and determine the accurate nucleic acid sequence of its genome. It would further be desirable to identify the mutations leading to cold  
20 adaptation, thus accurately characterizing the nucleic acid sequence of the ca master strain. It would also be desirable to produce and provide cold-adapted influenza strains through reassortment with currently circulating wild type strains. It would also be desirable to produce and use a cold-adapted influenza vaccine to prevent and/or treat influenza.

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#### SUMMARY OF THE INVENTION

The cold-adapted A/Ann Arbor/6/60 7PI (H2N2) influenza strain ("master strain") has been isolated and deposited, and its genome accurately sequenced and compared to its progenitor temperature-sensitive wild type E2(3) (wt 2(3)) virus. The A/Ann Arbor/6/60 virus is a single-stranded RNA virus having eight gene segments.  
30 During investigation of the virus leading to the vaccines of the present invention, unexpected deviations from previously reported sequences of the ca and wt were also identified. In particular, in the ca master strain sequences, seven nucleotide differences were found, occurring in the nucleoprotein gene (NP), the gene encoding an acidic polymerase protein (PA) and the gene encoding a basic polymerase polypeptide (PB2). ~~The wt progenitor strain and ca master strain have both been~~  
35 ~~deposited with the American Type Culture Collection, as set forth above.~~



In comparing the cold-adapted master strain to the wt progenitor strain, four nucleotide differences encoding two amino acid differences were found in three gene segments. Computer-predicted RNA folds projected different secondary structures between the cold-adapted and wild type molecules based on the two silent differences  
5 between them. Genes coding for the PA, matrix (M), and non-structural (NS) proteins were identical between the two viruses. The differences suggest that cold adaptation may serve to provide conformational changes in the RNA structure advantageous to growth at 25°C and provide a new form of genetic stability to the highly variable RNA genome.

10 With the identification of the correct nucleotide sequence of the ca master strain and its deposit, reassortant strains can now be produced which can be used as vaccines, to prophylactically and therapeutically treat influenza. Reassortant strains are produced by genetically combining the ca master strain with a variety of epidemic wild type viruses to yield reassortants which contain the hemagglutinin (HA) and  
15 neuraminidase (NA) gene segments of the wild type virus and the other six genome segments of the ca master strain. The reassortants thus contain the epidemic wild type strain genes that code for immunizing antigens found on the surface of the virus particle and the ca master strain genes that are responsible for the attenuated phenotype in humans and animals. To produce the vaccines of the present invention,  
20 a cold-adapted reassortant vaccine strain is passed once to prepare a virus seed lot which is used to produce vaccine pools.

In practicing the present invention, the amount of vaccine to be used or administered, alone or in combination with other agents, may vary with the patient being treated and may be monitored on a patient-by-patient basis by the physician.  
25 The vaccines of the present invention may also be administered in combination with other vaccines. Generally, a therapeutically effective amount of the vaccine will be administered for a therapeutically effective duration. By "therapeutically effective amount" and "therapeutically effective duration" is meant an amount and duration to achieve the desired therapeutic or prophylactic result in accordance with the present  
30 invention with medically acceptable side effects, which can be determined by those skilled in the medical arts.

The vaccines of the present invention may comprise the reassortant virus as well as a pharmaceutical formulation, together with a pharmaceutically acceptable carrier therefor. Each carrier must be "acceptable" in the sense of being compatible  
35 with the other ingredients of the formulation and not injurious to the patient. Formulations include those suitable for oral, nasal, topical (including transdermal,



buccal and sublingual), parenteral (including subcutaneous) and pulmonary administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy.

It will be appreciated that administration of the vaccines of the present invention will also be by procedures well-established in the pharmaceutical arts, e.g. preferably intranasally or orally, and most preferably intranasally. Intramuscular, intravenous and intradermal administration is also contemplated by the present invention, either alone or in combination.

The present invention thus comprises isolated nucleic and amino acids with sequences corresponding to the *ca* master and wild type strain sequences set forth in Sequence ID Listings 1-40. By "isolated" is meant substantially purified from the natural state through chemical, biochemical, immunological or other means, or obtained in substantially pure form by other methods known to those skilled in the art. By "substantially pure" is meant substantially free from undesirable contaminants such as other proteins. Thus, these terms are not meant to exclude synthetic and recombinant nucleic and amino acids which are contemplated within the scope of the present invention. These terms are also not meant to exclude nucleic and amino acids which are linked, bound or intentionally combined with other moieties such as transgenes, labels, flanking amino acid sequences and the like. It will also be appreciated that although the viruses of the present invention are RNA viruses, the present invention further includes DNA sequences corresponding and complementary thereto.

The present invention further comprises isolated or substantially pure *ca* master strain and wild type E2(3) A/AA/6/60 virus. By "isolated" or "substantially pure strain" is meant the viral strain substantially free from other contaminants such as other viruses, bacteria, and the like.

The present invention further comprises reassortant viruses produced by combining the cold-adapted master strain with a variety of epidemic wild type viruses. The two surface protein genes of an epidemic wild type virus are operatively-linked to the six internal genes of the cold-adapted master strain. By "operatively-linked" is meant attached or assembled in a manner which allows for expression of the surface and internal genes. In the context of reassortant viruses, operative linkage will allow for the packaging of the reassorted RNA into virions. It will also be appreciated that the term "gene" is used comprehensively to include all polynucleotide sequences coding for the gene product or protein, and is not limited to naturally occurring coding and regulating elements.



In addition, the present invention comprises the production and use of cold-adapted influenza vaccines to prevent and/or treat influenza.

Additional objects, advantages, and features of the present invention will become apparent from the following description and appended claims, taken in  
5 conjunction with the accompanying drawings and Sequence ID Listings.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and subjoined claims and by referencing the following drawings in which:

10 Figure 1 shows the derivation of the progenitor wild type and cold-adapted master strain A/AA/6/60 in PCK cells; and

Figure 2 shows the computer-projected RNA fold of cold-adapted and wild type 2(3) RNA1's (PB2's).

#### **DETAILED DESCRIPTION OF THE INVENTION**

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##### **OVERVIEW**

The nucleic and amino acid sequences for the eight genes of the cold-adapted master strain A/Ann Arbor/6/60 7PI (H2N2) are set forth in Sequence ID Listings 1-20. The nucleic and amino acid sequences for the eight genes of the wild type A/Ann Arbor/6/60 (H2N2) Egg Passage 2(3) are set forth in Sequence ID Listings 21-40.

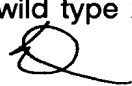
20 Table 1 summarizes the gene products of influenza A and B virus genes. The cold-adapted master strain and wild type 2(3) progenitor have been deposited with the ATCC, as described above. 



TABLE 1

Gene Products of Influenza A and B Viruses

5	<b>RNA</b>	<b>Gene Product(s)</b>	<b>Function</b>
	1	PB2	Viral polymerase component involved in RNA transcription
	2	PB1	Viral polymerase component with RNA transcription and replication activities
	3	PA	Viral polymerase component involved in RNA replication
	4	HA	Virion surface attachment and fusion glycoprotein, major antigenic determinant
10	5	NA	Virion surface glycoprotein with receptor-destroying enzyme activity, major antigenic determinant
	6	NP	Major nucleocapsid structural component and type-specific antigen
		NB	Glycoprotein putative membrane ion channel found only in type B
	7	M1	Membrane matrix protein and type-specific antigen
		M2	Nonglycosylated membrane ion channel, found only in type A
15	8	NS1	RNA-binding non-structural protein of transport function
		NS2	Cellular and virion protein of unknown function

The A/Ann Arbor/6/60 virus contains six internal genes, NS, M, NP, PA, basic polymerases (PB1 and PB2), and two surface genes, HA and NA. Seven nucleotide differences were found between the sequences of the present invention and those previously published for cold-adapted A/Ann Arbor/6/60: three in the NP gene, one in the PA gene and three in the PB2 gene. The eight viral genes and the discrepancies in the previously published sequences can be summarized as follows:

**NS.** The non-structural (NS) gene is the smallest RNA segment of influenza virus, 890 nucleotides long, and codes for the two non-structural proteins (NS1 and NS2) (nucleic acid Sequence ID Listing 1 and 3; amino acid Sequence ID Listings 2 and 4). There were no errors in the previously published sequences for the ca A/AA/6/60 NS1 and NS2 genes.



**M.** The matrix gene (M) is a 1,027 base nucleic acid sequence (nucleic acid Sequence ID Listings 5 and 7; amino acid Sequence ID Listings 6 and 8). There were also no errors in the previously published sequences for the ca A/AA/6/60 M gene.

**NP.** The nucleoprotein gene (NP) (nucleic acid Sequence ID Listing 9) is 1566  
5 nucleotides in length and encodes a basic structural protein of 498 amino acid residues (amino acid Sequence ID Listing 10) which specifically interacts with RNA molecules to form ribonucleoprotein complexes and has sequences that direct its migration into the nuclei of infected cells. Despite previous reports, nucleotide 627 of NP is actually cytosine not adenine, and nucleotide 909 is guanine, not cytosine. In  
10 addition, nucleotide 113 was previously published as an adenine, although in GenBank it is reported as a cytosine. Cox, N.J. et al., *Viol.* 167:554-567 (1988). Regardless of this discrepancy, it is now known that nucleotide 113 is actually a cytosine.

**PA.** The polymerase acidic protein gene (PA) RNA sequence (nucleic acid  
15 Sequence ID Listing 11) is 2233 nucleotides in length and encodes an acidic polymerase protein 716 amino acids in length (amino acid Sequence ID Listing 12). Although previous publications indicate thymine at nucleotide 75 of PA, guanine is actually present at that position.

**PB1.** The polymerase basic 1 gene (PB1) RNA sequence (nucleic acid  
20 Sequence ID Listing 13) is 2341 nucleotides in length and encodes a basic polymerase protein 757 amino acids in length (amino acid Sequence ID Listing 14). No errors in the previously published sequence were found.

**PB2.** The polymerase basic 2 gene (PB2) RNA sequence (nucleic acid  
Sequence ID Listing 15) is 2341 nucleotides in length and encodes a basic  
25 polymerase polypeptide of 759 amino acids (amino acid Sequence ID Listing 16). There are three errors in the previously published sequence for PB2: thymine at 714 instead of the previously published cytosine at that position; guanine at 936 instead of adenine; and cytosine instead of thymine is the predominant base at 1933, with thymine as the secondary base.

**HA and NA.** The hemagglutinin gene (HA) and neuraminidase gene (NA) code  
30 for surface receptors. HA is 1773 nucleotides long and codes for a 562 amino acid sequence (nucleic acid Sequence ID Listing 17; amino acid Sequence ID Listing 18). See Schäfer, J.R. et al. *Viol.* 194:781-788 (1993). NA is 1467 nucleotides long and codes for a 469 amino acid sequence (nucleic acid Sequence ID Listing 19; amino  
35 acid Sequence ID Listing 20).



Results from previous studies indicate that cold adaptation causes mutations in every gene of the A/AA/6/60 master strain, thus ensuring the genetic stability of the virus. There are actually, however, four base differences in three of the internal genes of A/AA/6/60 after 28 passages in primary chicken kidney (PCK) cells and four passages in eggs. Two of the substituted bases are silent and two result in single amino acid differences in two of the genes. Moreover, the wt 2(3) progenitor virus is attenuated in ferrets. Hence, the stability and immunogenicity of the ca A/AA/6/60 vaccine appears to reflect inherent properties of the wt A/AA/6/60 E2(3) virus selected as the progenitor for the master strain. This interpretation is supported by the large number of amino acids unique to both wt 2(3) and ca viruses (see Table 3), some of which may be attenuating.

By attempting to identify changes arising from cold adaptation using the ca master strain and the wt 2(3) virus, there is at least one further critical variable – passage of the virus in different host tissues. It has been shown that the host cell influences the selection of antigenic variants of influenza viruses. Katz, J.M., et al., *Virology* 156:386-395 (1987). In studies of the HA gene of H3N2 viruses, passage in Madin-Darby canine kidney (MDCK) cells and in primary chick kidney (PCK) cells selected populations that were homogeneous and true to the original isolate for this gene whereas passage in eggs selected heterogeneous populations. Katz, J.M., et al., *J. Gen. Virol.* 73:1159-1165 (1992). Thus, the changes observed could relate to the number of passages of each virus. The wild type 2(3) virus, with only two egg passages, is the only virus among all of those listed in GenBank to have isoleucine encoded by base 1276 of RNA2 and asparagine encoded by base 113 of NP. The positions of those two amino acids in the cold-adapted virus, with 29 PCK passages and 4 egg passages, are the same as those of all other viruses listed in GenBank. This finding suggests that the valine encoded by base 1276 in the cold-adapted PB1 is a host adaptation change rather than a cold adaptation change; the same holds for the threonine encoded by base 113 of the cold-adapted NP gene.

Differences between the wt 2(3) sequence as set forth herein and the wt 28-32 previously sequenced reflect mutations acquired during high passage in eggs at 35°C. Cox, N.J., et al., *Virology* 167:554-567 (1988). These mutations may be the result of host adaptation in the egg or simply selection of the highly variable RNA population with the highest relative fitness. Clarke, D.K., et al., *J. Virol.* 67:222-228 (1993).

Since only the ca RNA1 has guanine (G) at position 141 and cytosine (C) at position 1933, by comparison with all other human RNA1's in GenBank, the two base changes between the wt 2(3) and ca RNA1's may in fact be cold-adapted changes.



No wild type human viruses, including the wt 2(3) progenitor, have G at 141 or C at 1933. This suggests that cold adaptation may operate at the RNA level. Recent findings indicate that unique RNA structures in influenza viruses may have common regulatory functions. Parvin, J.D., et al., *J. Virol.* 63:5142-5149 (1989). The more  
5 stable conformation of the ca molecule predicted by base pairing might provide a growth advantage over the predicted conformation of the wt 2(3) molecule. The importance of RNA structure to biological function has been well documented for poliovirus. Racaniello, V.R., et al., *Virology* 155:498-507 (1986). The presence of a hairpin structure at the 5' noncoding end has been shown to be necessary for the ts  
10 phenotype of the virus.

Although RNA viruses have notoriously high mutation rates and have been referred to as "quasi-species," Holland, J.J., et al., *Cur. Topics Microbiol. Immunol.* (Springer-Verlag) 176:1-20 (1992), the ca A/AA/6/60 virus showed unusual stability after cold adaptation in PCK cells. In 33 passages there were only four sequence  
15 changes in the six internal genes, yielding a mutation rate of  $2 \times 10^{-6}$ . Compared to expected chance mutation rates calculated for the NS gene in MDCK cells, one would have expected 21 sequence changes. Parvin, J.D., et al., *J. Virol.* 59(2):377-383 (1986). Since RNA viruses have not been shown to have proofreading functions, this low mutation rate may be an inherent property of the wild type polymerases or a result  
20 of the cold adaptation process, or both. Suarez has shown that wild type viruses comprise subgroups with different mutation rates. Suarez, P., et al., *J. Virol.* 66(4):2491-2494 (1992). The wt A/AA/6/60 may have a dominant population with a more error-free polymerase. In addition, certain positions may simply be difficult for the polymerase to read, owing to conformation of the RNA molecule. Lowering the  
25 growth temperature by 10°C slows the whole replicative process including the speed at which the polymerase unit is moving. *Thermus aquaticus* (Taq) polymerase is notorious for its high error rate due in part to the high temperature of its use, and it has been shown that a 5°C reduction in temperature increases the fidelity of *Tub* polymerase. Kainz, P., et al., *Anal. Biochem.* 202:46-49 (1992). The lower temperature  
30 may provide a slowed-down environment conducive to faithful copying even in areas with conformational bends and twists. Thus the A/AA/6/60 polymerase might exhibit greater fidelity at 25°C than at 35°C.

Single gene cold-adapted reassortants, constructed to identify the genetic basis of the ca and ts phenotypes and of attenuation, should be interpreted with care.  
35 For instance, in the study by Snyder et al., conducted in a background of A/Korea/1/82 genes, both PA and M were implicated in attenuation. Snyder, M.H., et



al., *J. Virol.* 62(2):488-495 (1988). Neither gene showed sequence differences from its wt 2(3) counterpart in the present analysis. This would suggest that single gene wt 2(3) PA or wt 2(3) M in an A/Korea background would react similarly to the ca PA and M single genes. From the sequence data, one would also expect that RNA1  
5 encoding PB2 would contribute to the ca phenotype in single gene studies and yet only PA was involved. Snyder, M.H., et al., *J. Virol.* 62(2):488-495 (1988). Gene constellation studies suggest that single gene studies in one wild type may be applicable to only that wild type. Subbarao, E.K., et al., *Virus Res.* 25:37-50 (1992). In a different wild type background, the assignment of phenotype to specific ca genes  
10 might change because other wild type genes might be dominant or carry natural extragenic suppressor mutations. This emphasizes the need for the presence of six genes from the ca virus rather than five in ca reassortants to ensure maximum stability. Maassab, H.F., et al., *J. Infect. Dis.* 146(6):780-790 (1982).

#### SPECIFIC EXAMPLE 1 - SEQUENCING

##### 15 A. MATERIALS AND METHODS

**Viruses.** All viruses were supplied by Professor H.F. Maassab at the University of Michigan and the ca master strain and wild type progenitor strain viruses have now been deposited with the ATCC as previously set forth. Steps in the preparation of the ca master strain A/AA/6/60 7PI (H2N2) live influenza virus and the wt A/AA/6/60  
20 (H2N2) egg passage 2(3) virus are shown in Figure 1. In Figure 1, PCK cells refers to primary chick kidney cells, SPAFAS refers to specific pathogen-free eggs and PI refers to plaque-purified. To guard against any possibility of mix-up in the two viruses, the passage history of both viruses was carefully traced and their separate identities were verified. Moreover, the two viruses were grown in different institutions and  
25 sequenced separately. The authenticity of the wt A/AA/6/60 E2(3) virus is supported by sequence differences between the HA's and NA's of the cold-adapted and wild type viruses. Viruses grown in 11-day old embryonated chicken eggs and virion RNA were prepared as previously described. Bean, W.J., et al., *Anal. Biochem.* 102:228-232 (1980).

30 **Growth and Infectivity of Viruses.** Plaque titrations were performed with both viruses in PCK cells at 25°C, 33°C, and 39°C, and in MDCK cells at 33°C and 39°C. Mills, J., et al., *J. Infect. Dis.* 123:145-157 (1971). Plaque counts obtained at each of the three temperatures were compared to assess the ca and ts phenotypes of both viruses.

35 **Ferret Studies.** One week before infection with virus, 4 female ferrets were bled and screened for influenza antibody against A/Taiwan/1/86 (H1N1),



A/Beijing/353/89 (H3N2), wt A/AA/6/60 (H2N2) E2(3) and B/Victoria/2/87. The animals' temperatures were taken twice a day for 1 week preceding their inoculation with  $1 \times 10^9$  EID<sub>50</sub> of wt E2(3), and then until they were sacrificed at either 3 or 8 days after infection. Lungs and turbinates of the ferrets were examined by previously reported  
5 methods. Maassab, H.F., et al., *J. Infect. Dis.* 146(6):780-790 (1982).

**Gene Cloning.** Double-stranded cDNA was prepared as previously described. Huddleston, J.A., et al., *Nucleic Acids Res.* 10:1029-1039 (1982). Full-length double-stranded copies of genes 4 through 8 (HA, NA, NP, M, NS) were blunt-end ligated into the Pvu II site of vector Pvu II, obtained from C. Naeve at St. Jude Children's Research  
10 Hospital.

For the polymerase genes (PB1, PB2, PA), the first-strand cDNA was amplified by the polymerase chain reaction (PCR) using phosphorylated primers. "Gene-cleaned" PCR product was blunt-end ligated into the Pvu II site of pATX.

**Nucleic Acid Sequencing.** Nucleotides of all eight cloned genes of each virus  
15 were sequenced by the method of Chen and Seeburg using alkali-denatured DNA templates. Chen, E.Y., et al., *DNA* 4:165-170 (1985). Due to the extreme heterogeneity of RNA viruses, several clones of each gene were sequenced to avoid reporting the sequence of a minor mutant population. Clones of each orientation were sequenced for each gene. If the two clones differed at any position, as many as 7  
20 clones of each gene were sequenced and the consensus sequence was reported. Compressions were resolved by the addition of 42% formamide to the gels.

Differences between the cold-adapted virus and the wild type E2(3) virus were confirmed by direct sequencing of the virion RNA, a method which would expose any mutations introduced by use of the *Taq* polymerase. Air, G.M. *Virology* 97:468-472  
25 (1979).

**Sequence Analysis.** The IntelliGenetics software package (Palo Alto, CA) was used to analyze nucleotide sequence data. Chou-Fasman two-dimensional protein structure predictions were made with programs available at the St. Jude Molecular Biology Computing Center. The reliability of protein folding by this method is  
30 predicted to be approximately 60%. Fasman, G.D. "Prediction of Protein Structure and the Principles of Protein Confirmation" (Plenum, New York), p. 417-467 (1986).

The Zuker Fold program on the Cray Y-MP supercomputer at the Pittsburgh Supercomputing Center was used to study the folding of RNA molecules. Optimal foldings were obtained using the Zuker algorithm which calculates the structure  
35 exhibiting minimal free energy. Zuker, M., et al., *Nucleic Acids Res.* 9:133-148 (1981). This program calculates the structure that is energetically most favorable and has a



predicted accuracy of 80%, although the structure with the lowest free energy may not represent all biologically active structures. Zuker, M., et al., *Nucleic Acids Res.* 9:133-148 (1981).

## B. RESULTS

5        **Biological Properties.** The *ca* and *ts* characteristics of the viruses in PCK cells was first examined. The *ca* master strain reached essentially the same titer at 25°C ( $3.0 \times 10^8$ ) as it did at 33°C, but failed to grow at 39°C (see Table 2), fulfilling accepted criteria for cold adaptation and temperature sensitivity. By contrast, on day 6, the *wt* E2(3) virus had produced fewer than  $1.0 \times 10^5$  plaques at 25°C, although by  
10 day 8 it had generated  $5.0 \times 10^6$  plaques, indicating a subpopulation of virus capable of growth at low temperatures. The 4-log reduction in growth at 39°C compared with that at 33°C demonstrates the *ts* phenotype of the *wt* 2(3) virus. Similar results were obtained in MDCK cells at 33°C and 30°C (data not shown).

The pathogenicity of the wild type 2(3) virus was studied in ferrets. The virus  
15 was not recovered from lung tissue in any of the 4 animals examined, and it was recovered from turbinates in only the 2 animals sacrificed on day 3 (data not shown). None of the ferrets showed physical signs of illness, such as coryza, lethargy or sneezing. Rises in temperature ranging from 1°C to 1.5°C were observed, but they persisted for only several hours and were not considered significant since normal  
20 temperatures fluctuated by 1°C. These results, which correspond to findings with the *ca* virus, indicate that the *wt* 2(3) virus was attenuated before cold adaptation. Maassab, H.F., et al., *J. Infect. Dis.* 146(6):780-790 (1982).



**TABLE 2**  
**Infectivity Titers of A/AA/6/60 (H2N2)**

Virus	Number of Plaques in Primary Chick Kidney Cells <sup>a</sup>		
	33°C <sup>b</sup>	39°C <sup>b</sup>	25°C
ca Master Strain A/AA/6/60 (H2N2) 7PI (SE4)	6.0 x 10 <sup>8</sup>	<1.0 x 10 <sup>4</sup>	5.0 x 10 <sup>7</sup> on day 6 <sup>c</sup>
			8.0 x 10 <sup>7</sup> on day 7
			3.0 x 10 <sup>8</sup> on day 8
wt A/AA/6/60 (H2N2) E2(3)	1.5 x 10 <sup>8</sup>	2.0 x 10 <sup>4</sup>	<1.0 x 10 <sup>5</sup> on day 6
			8.0 x 10 <sup>5</sup> on day 7
			5.0 x 10 <sup>6</sup> on day 8

<sup>a</sup>Similar results were obtained in MDCK cells at 33°C and 39°C.

<sup>b</sup>Infectivity titers at 33°C and 39°C were determined on post-infection day 4.

<sup>c</sup>Post-infection days.

Tests were also performed employing ferrets to determine whether the cold-adapted vaccine would interfere with or block growth of the influenza virus. The experimental protocol and results of this study are set forth in U.S. Patent No. 5,149,531, issued September 22, 1992 to Younger et al., hereby incorporated by reference.

**Sequencing.** Table 3 compares sequencing results of the ca master strain with wt E2(3) virus. The data represent consensus DNA sequencing of multiple clones. If the clone consensus indicated a difference between the two viruses, RNA sequence data were used to support the findings. Positions reported as mixed populations in Table 3 show the distribution of the clones.

Between the internal genes of the ca and the wt 2(3) viruses, no differences were found in the genes coding for PA, M or NS, even though PA and M were previously reported to be important for attenuation of the ca master strain and cold adaptation was attributed to PA. Snyder, M.H., et al., *J. Virol.* 62(2):488-495 (1988). Differences were found in the genes coding for PB2, PB1 and NP.



TABLE 3

Sequence Differences between *wt* 2(3) and *ca* A/Ann Arbor/6/60 Viruses

Gene	Base No.	Amino Acid No.	<i>wt</i> A/AA/6/60 E2		<i>ca</i> A/AA/6/60	
			Base	Amino Acid	Base	Amino Acid
PB2	141		A/g(4/2)		G (5)	
	1933		T/c(4/2)		C/t(4/1)	
PB1	1276	418	A (5)	Ile	G/a(4/3)	Val
PA			-	-	-	-
HA	144	34	A (2)	Asn	T (2)	Ile
	455	138	G (2)	Ala	A (2)	Thr
	729	229	A (2)	Lys	C (2)	Thr
NA	394		C (2)		T (4)	
	604		A (2)		T (4)	
NP	113	23	A/c(2/1)	Asn	C/a(3/1)	Thr
M			-	-	-	-
NS			-	-	-	-

In Table 3 above, in positions with mixed bases, the capital letter represents the dominant base. The distribution of the clones representing the positions with differences between the *wt* 2(3) and the *ca* internal genes are shown next to the bases.

**RNA1 (PB2).** Two nucleotide differences, in bases 141 and 1933, were found between the *ca* and *wt* 2(3) RNA1 genes, which encode a basic polymerase protein 759 amino acids in length. Called PB2, this protein is part of the transcriptase complex and has been identified as recognizing and binding the cap structure of the host-cell primer RNA. Plotch, S.J., et al., *Cell* 23:847-858 (1981). Both changes are in the coding region but are silent. Moreover, bases 141 and 1933 of the *ca* RNA1 are unique among all other human RNA1 sequences in GenBank. Position 1933 in the *wt* 2(3) and *ca* RNA1 segments is a mixed population of two bases; however, the darker band in the RNA sequence (thymine (T) in *wt* 2(3) and cytosine (C) in *ca*) conforms with the consensus DNA sequence reported in Table 3.

To assess the potential functional significance of the two nucleotide sequence differences between the *ca* and the *wt* 2(3) viruses, the Zuker RNA-fold algorithm and computer modeling techniques were used to predict RNA secondary structures. As



shown in Figure 2, the difference at base 141 does not impinge on the predicted structure of RNA1 because it is part of an unpaired loop in both molecules; however, the change at nucleotide 1933, T in *wt* 2(3) to C in *ca* (shown by arrows in Figure 2), does affect the predicted fold of RNA1. The RNA fold of the *ca* virus has greater  
5 stability than the analogous fold of *wt* 2(3), as judged by its lower free energy of -736.2 compared to -733.6 for the *wt* 2(3) molecule. Both folds were pivoted -25° at pair 1068/1381 and 180° at pair 1675/1861 to better visualize the area of difference between the two molecules. The single base change at 1933 causes a cascade of 163 pairing differences, from base 1888 to base 2151, and thus might constitute a true  
10 cold adaptation. Similar RNA1 sequencing results were obtained for a *wt* A/AA/6/60 E3(4) passage virus.

**RNA2 (PB1).** The only nucleotide change found between the RNA2 genes of the *ca* and *wt* 2(3) viruses occurred at base 1276, resulting in a substitution of valine (*ca*) for isoleucine (*wt* 2(3)), both of which are hydrophobic and uncharged. RNA2  
15 encodes a basic polymerase (PB1) that mediates transcription and elongation of the mRNA chain. Braam, J., et al., *Cell* 34:609-618 (1983). Analysis of protein secondary structures predicted by Chou-Fasman and Garnier-Osguthorpe methods, as well as computer-predicted RNA structures, failed to reveal differences between the *ca* and *wt* 2(3) RNA2's. Valine is not an amino acid unique to the *ca* virus because later  
20 passages of the *wt* A/AA/6/60 virus (both *wt* E6 and *wt* E28) also have valine at this position, as do all other RNA2's in GenBank. Both DNA clones and RNA sequencing show that base 1276 comprises a mixed population of adenine (A) and guanine (G) in the *ca* RNA2; however, the G predominates.

**RNA6 (NP).** The nucleoprotein gene (RNA6) encodes a basic protein 498  
25 amino acids in length which specifically interacts with RNA molecules to form ribonucleoprotein complexes. Huddleston, J.A., et al., *Nucleic Acids Res.* 10:1029-1039 (1982). NP is necessary for transcription and is a major determinant of host range. Huang, T.S., et al., *J. Virol.* 64:5669-5 673 (1990); Scholtissek, C., et al., *Virology* 147:287-294 (1985). There was one difference between the *wt* 2(3) and the *ca* NP  
30 molecules, at base 113 leading to substitution of threonine for asparagine, neither of which is hydrophobic or charged. The reverse change was reported in Cox, N.J., et al., *Virology* 167:554-567 (1988).

Although having similar protein secondary structures by Chou-Fasman and Garnier-Osguthorpe predictions, the two RNA molecules showed a distinct difference  
35 in their predicted RNA structures. In *wt* 2(3) RNA6, base 113 creates a larger unpaired loop making the molecule less stable than *ca* RNA6 (structure not shown). DNA



cloning and RNA sequencing revealed that base 113 is a mixed population of A and C in both the *wt* 2(3) and the *ca* RNA6's; however, in the *wt* 2(3) the consensus base is A and in the *ca* the consensus base is C.

5 The asparagine in the *wt* 2(3) virus is unique among all reported NP molecules (see Table 3), but not the threonine of the *ca* virus. The A/AA/6/60 (*wt* and *ca*) viruses are the only viruses in 54 GenBank sequences with an inserted A at base 1550 near the putative polyadenylation signal.

**RNA4 (HA) and RNA5 (NA).** The sequences of *ca* RNA4 (HA) and *ca* RNA5 (NA) have not been previously reported, as neither molecule is included in *ca* reassortant vaccines. RNA4 encodes the hemagglutinin (HA) surface glycoprotein (562 amino acids in length), while RNA5, encodes the neuraminidase (NA) surface glycoprotein (469 amino acids in length). Two silent nucleotide differences were observed between *ca* RNA5 and *wt* 2(3) RNA5 at bases 394 and 604. Three additional differences seen at bases 144, 455, and 729 of *ca* RNA4 and *wt* 2(3) RNA4  
10 coded for amino acid changes: asparagine to isoleucine (position 34), alanine to threonine (position 138) and lysine to threonine (position 229). The presence of clear differences in these two surface genes underscores the different passage histories of the two viruses and provides additional evidence for their separate identities.

## **SPECIFIC EXAMPLE 2 - SEQUENCE COMPARISONS**

20 **Sequence of Wild Type Progenitor.** Table 4 presents positions for each gene where the *ca* and *wt* 2(3) viruses have unique amino acids, by comparison to previous GenBank sequences. Webster, R.G., et al., *Microbiol. Rev.* 56(1):152-179 (1992). In Table 5, a comparison to data previously published is shown and differences between the *wt* 2(3) and *ca* sequences as set forth herein, and the previously published  
25 sequences, are shown in bold type and bracketed. In positions with mixed bases, the capital letter represents the predominant base. Some of these amino acids found only in the two *ts* A/AA/6/60 viruses may be attenuating. However, many of the viruses reported in GenBank have been extensively passaged in the laboratory and will have accumulated mutations related to high relative fitness and host adaptation.  
30 Comparison to the A/AA/6/60 *wt* 28 virus previously sequenced provides further insight into attenuating lesions. Cox, N.J., et al., *Virology* 167:554-567 (1988).



TABLE 4

Unique Amino Acid Differences between Temperature-sensitive and Attenuated wt 2(3) and ca A/AA/6/60 Viruses and Other Influenza Viruses in GenBank

Gene	No. in GenBank	Base No.	A/AA/6/60		GenBank Viruses <sup>b</sup>
			ca/wt 2(3)	wt 28	
PB2 <sup>a</sup>	27	821	Ser	Asn	Asn
		954	Glu	Glu	Asp
PB1	23	215	His	His	Pro
		1096	Lys	Lys	Glu
		1276	Val/Ile	Val	Val
		1395	Asp	Glu	Glu
		1660	Leu	Leu	Met
PA	21	599	His	His	Arg
		2167/8	Pro	Leu	Leu
NP	54	113	Thr/Asn	Thr	Thr
		1550	A	-	-
M1	44	453	Val	Val	Ala
		457	Leu	Leu	Phe
		678/9	Val	Val	Ala
M2	44	847	His	His	Arg
		969	Ser	Ala	Ala
NS1	73	35	Pro	Pro	Ser
		483	Thr	Ala	Glu

<sup>a</sup> Five other silent differences.

<sup>b</sup> Sources of GenBank viruses for each gene used in phylogenetic analysis are reported in Webster R.G., et al., *Microbiol. Rev.* 56(1):152-179 (1992).



TABLE 5

Summary of Comparative Sequence Data for A/Ann Arbor/6/60  
Wild Type and Cold-Adapted Viruses

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Data from Study						Data Previously Published <sup>a</sup>			
wt A/AA/6/60 E2(3)				ca A/AA/6/60		ca A/AA/6/60		wt A/AA/6/60 E28	
Gene	Base No.	Base	AA	Base	AA	Base	AA	Base	AA
PB2	141 <sup>+</sup>	A/g		G		G		A	
	426	C		C		C		T	
	714	T		[T]		[C]		[C]	
	821	G 265	ser	G	ser	G	ser	A	as p
	963	G		[G]		[A]		[A]	
	1182	T		T		T		A	
	1212	T		T		T		C	
	1353	G		G		G		T	
	1923	G		G		G		A	
	1933 <sup>~</sup>	T/c		[C]/t		[T]		T	
PB1	123	G		G		G		A	
	486	T		T		T		C	
	1195	G 391	glu	G	glu	G	glu	A	lys
	1276 <sup>^</sup>	A/g 418	ile	G/a	val	G	val	G	val
	1395	T 457	asp	T	asp	T	asp	G	glu
	1766	G 581	gly	G	gly	G	gly	A	glu
	2005	A 661	thr	A	thr	A	thr	G	ala
PA	20	C		C		C		T	
	75	G		[G]		[T]		[T]	
	1861	G 613	glu	G	glu	G	glu	A	lys
	2167	C 715	pro	C	pro	C	pro	T	leu
	2168	C		C		C		T	
HA	144	A 34	asn	T	ile				



Data from Study						Data Previously Published <sup>a</sup>			
wt A/AA/6/60 E2(3)				ca A/AA/6/60		ca A/AA/6/60		wt A/AA/6/60 E28	
Gene	Base No.	Base	AA	Base	AA	Base	AA	Base	AA
	455	G	138	ala	A	thr			
	729	A	229	lys	C	thr			
NA	394	C		T					
	604	A		T					
5 NP	113 <sup>&lt;</sup>	A/c	asn	C/[a]	thr	[A]	asn	C	thr
		23							
	146	G	34	gly	G	gly	G	gly	A
									p
	627	C		[C]				A	
	909	G		[G]				C	
	1550	A		A		A		-	
10 M	969	T	ser	T	ser	T	ser	G	ala
NS	483	A	153	thr	A	thr	A	thr	G
									ala
	813	G		G		G		A	

<sup>a</sup> Cox, N.J., et al., *Virology* 167: 554-567 (1988).

15

The distribution of the clones representing the positions with the differences between the wt 2(3) and the ca viruses are listed below:

<sup>+</sup> wt 2(3) PB2 141 four clones A, two clones G  
ca PB2 141 five clones G

20

<sup>~</sup> wt 2(3) PB2 1933 four clones T, two clones C  
ca PB2 1933 four clones C, one clone T

<sup>^</sup> wt 2(3) PB1 1276 five clones A  
ca PB1 1276 four clones G, three clones A

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<sup><</sup> wt 2(3) NP 113 two clones A, one clone C  
ca NP 113 three clones C, one clone A

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### SPECIFIC EXAMPLE 3 - REASSORTANT SCHEMES

#### A. TYPE A REASSORTANTS

The following is a procedure for developing Type A 6/2 cold-adapted influenza virus vaccine (CAIV) reassortants.

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#### Materials

**Media.** The media used in this sample were prepared using the following components: a) HBSS - 500 ml HBSS (BioWhitaker 10-508); 0.5 ml gentamicin sulfate 50 mg/ml (BioWhitaker 17-518); and adjust pH to 7.0 using 0.5N NaOH; b) 2 x Eagle's - 500 ml HBSS (BioWhitaker 10-508); 10 ml BME amino acids (GIBCO 320-1051); 10 ml BME vitamins (GIBCO 320-1040); 10 ml L-glutamine (GIBCO 320-5030); and 0.5 ml gentamicin sulfate 50 mg/ml (BioWhitaker 17-518); adjust pH to 7.0 using 0.5N NaOH; c) 0.5N NaOH - 2 g NaOH; 100 ml Type I deionized water; sterilize by autoclaving 250°C for 15 min, liquid cycle.

**Inoculum.** Inocula were prepared as follows: Cold-adapted Master Strain Parent (A/Ann Arbor/6/60 - 7PI) - make a  $10^{-2}$  dilution in 2 x Eagle's. Wild Type Parent - make a  $10^{-1}$  dilution in 2 x Eagle's. Combine equal volumes of the two diluted parents (1:1 dilution) and use this as the inoculum.

**Cells.** Use SPAFAS-derived primary chick kidney (SPF-PCK) cells grown in 16 x 125 mm tissue culture tubes on the fifth day after seeding.

20

#### Passages

**SPF-CK1 Passage.** SPF-CK1 passages were performed as follows: 1) remove growth media from ten SPF-PCK tubes; 2) wash SPF-PCK tubes with 1 ml of HBSS media; 3) inoculate with 0.3 ml of inoculum per tube; 4) adsorb at room temperature for 90 min while continuously rocking at low speed; 5) remove inoculum; 6) wash SPF-PCK tubes with 1 ml of HBSS media; 7) add 1 ml of 2 x Eagle's media and incubate at 33°C; 8) after 24 hr feed tubes with 0.3 ml of 2 x Eagle's media; and 9) observe cells daily for cytopathic effect (CPE). When CPE is >75%, pass the tubes to CK2 (usually 48-72 hr).

**SPF-CK2 Passage.** SPF-CK2 passages were performed as follows: 1) remove growth media from the SPF-PCK tubes; 2) wash SPF-PCK tubes with 1 ml of HBSS media; 3) serially pass the CK1 passage with 0.3 ml of inoculum per tube; 4) adsorb at room temperature for 90 min while continuously rocking at low speed; 5) remove inoculum; 6) wash SPF-PCK tubes with 1 ml of HBSS media; 7) add 0.3 ml of ferret antisera against A/AA/6/60-7PI which has been treated by the trypsin-periodate method to remove nonspecific inhibitors which has been filter sterilized (0.22 $\mu$ ). Use a 1:32 - 1:56 final dilution of sera (note that the treated sera is a 1:8 dilution); 8)



adsorb at room temperature for 15 min while continuously rocking at low speed; 9) add 1 ml of 2 x Eagle's media and incubate at 33°C; and 10) observe cells daily for CPE. When CPE is >75%, pass the tubes to CK3 (usually 48-72 hr).

**SPF-CK3 Passage.** The procedure for this passage was identical to the CK2 passage. When the CPE of this passage is >75%, plaque-purify the material in SPF-PCK cells.

### Plaque Purification/Genotype Screening

**1PI (1st) Plaque Purification.** First plaque purification and genotype screening were performed as follows: 1) serially dilute the CK3 passage in 2 x Eagle's media through a  $10^{-4}$  dilution, one ml of each dilution is needed per flask infected; 2) plaque the  $10^{-3}$  and  $10^{-4}$  dilution of each tube at 33°C following the procedure for plaquing in PCK cells; 3) pick several plaques for each tube. Using a sterile cotton plugged Pasteur pipet which has been bent to a 90° angle remove the agar and cells surrounding a well-isolated plaque. Draw a small volume of HBSS into the Pasteur pipet prior to picking the plaque to facilitate the expulsion of the plaque from the Pasteur pipet. Transfer the plaque material to a sterile capped tube containing 0.5 ml of 2 x Eagle's media. One plaque from each tube is passed in SPAFAS eggs and the other plaques should be frozen at -70°C as backup material; 4) pass one plaque in two SPAFAS eggs (0.2 ml of inoculum per egg) at 33°C for 72 hr. Refrigerate eggs at 4°C for at least one hr prior to harvesting the allantoic fluid. Determine the hemagglutinin titer (HA) of the egg pool to confirm the presence of virus and determine plaquing dilutions for the next purification. Two eggs will provide all the virus needed; and 5) genotype the 1PI egg material following the genotype procedure to identify potential 6/2 candidates.

**2PI (2nd) Plaque Purification.** Second plaque purification and genotype screening were performed as follows: 1) plaque the 1PI egg material in SPF-PCK cells at 33°C following the procedure for plaquing in PCK cells, using the following appropriate dilutions to obtain well-isolated plaques:

TABLE 6

HA Titers	Approximate Dilutions
< 1:32	$10^{-3}$ and $10^{-4}$
$\leq$ 1:128	$10^{-4}$ and $10^{-5}$
$\leq$ 1:512	$10^{-5}$ and $10^{-6}$
> 1:512	$10^{-5}$ , $10^{-6}$ and $10^{-7}$



2PI plaques should be derived from the same material which is genotyped since the egg passage may exert selective pressure on the plaques; and 2) pick several plaques following the procedure previously described. One plaque from each tube will be replaques in SPF-PCK cells and the other plaques should be frozen at -70°C as backup material.

**3PI (3rd) Plaque Purification.** Third plaque purification and genotype screening were performed as follows: 1) plaque the 2PI plaques in SPF-PCK cells at 33°C following the procedure for plaquing in PCK cells. The appropriate dilutions for this passage are  $10^{-1}$  and  $10^{-2}$ ; 2) pick several plaques following the procedure previously described. At this time you should know which are potential 6/2's and non-candidates can be discarded. One plaque will be amplified in SPAFAS eggs at 33°C and the others should be frozen at -70°C as backup material; 3) genotype the 6/2 candidates to confirm that 3PI passages have the 6/2 gene configuration; and 4) characterize the phenotypic profile of the 6/2 vaccine candidates at 25°C, 33°C and 39°C to confirm the presence of the *ca* and *ts* markers.

#### **B. TYPE B REASSORTANTS**

The following is a procedure for developing Type B 6/2 cold-adapted influenza virus vaccine (CAIV) reassortants.

##### **Materials**

**Media.** The media used in part B of this example were prepared as described in part A above.

**Inocula.** Inocula of the *ca* master strain parent and wild type are diluted to  $10^{-2}$ .

**Cells.** SPAFAS primary chick kidney (SPF-PCK) were grown as described in part A above.

##### **Passages**

**SPF-CK1 Passage.** SPF-CK1 passages were performed as follows: 1) remove growth media from ten SPF-PCK tubes; 2) wash SPF-PCK tubes with 1 ml of HBSS media; 3) inoculate with 0.3 ml of inoculum per tube; 4) adsorb at room temperature for 90 min while continuously rocking at low speed; 5) remove inoculum; 6) wash SPF-PCK tubes with 1 ml of HBSS media; 7) add 0.3 ml of ferret antisera against B/AA/1/66 CL 4-1-7PI treated by the trypsin-periodate method to remove nonspecific inhibitors and filter sterilized ( $0.22\mu$ ). Use a 1:56 final dilution of sera (note that the treated sera is a 1:8 dilution); 8) adsorb at room temperature for 15 min while continuously rocking at low speed; 9) add 1 ml of 2 x Eagle's media and incubate at 25°C; and 10) observe



cells daily for cytopathic effect (CPE). When CPE is >75%, pass the tubes to CK2 (usually 72-96 hr).

**SPF-CK2 Passage.** SPF-CK2 passages were performed as follows: 1) remove growth media from the SPF-PCK tubes; 2) wash SPF-PCK tubes with 1 ml of HBSS media; 3) serially pass the CK1 passage with 0.3 ml of inoculum per tube; 4) adsorb at room temperature for 90 min while continuously rocking at low speed; 5) remove inoculum; 6) wash SPF-PCK tubes with 1 ml of HBSS media; 7) add 0.3 ml of ferret antisera against B/AA/1/66 CL 4-1-7PI treated by the trypsin-periodate method to remove nonspecific inhibitors which has been filter sterilized (0.22 $\mu$ ). Use a 1:56 final dilution of sera (note that the treated sera is a 1:8 dilution); 8) adsorb at room temperature for 15 min while continuously rocking at low speed; 9) add 1 ml of 2 x Eagle's media and incubate at 33°C; and 10) observe cells daily for CPE. When CPE is >75%, pass the tubes to CK3 (usually 48-72 hr).

#### Plaque Purification/Genotype Screening

**1PI (1st) Plaque Purification.** First plaque purifications and genotype screening were performed as follows: 1) serially dilute the CK2 passage in 2 x Eagle's media through a  $10^{-4}$  dilution, one ml of each dilution is needed per flask infected; 2) plaque the  $10^{-3}$  and  $10^{-4}$  dilution of each tube at 33°C following the procedure for plaquing in PCK cells; 3) pick several plaques for each tube. Using a sterile, cotton-plugged Pasteur pipet which has been bent to a 90° angle, remove the agar and cells surrounding a well-isolated plaque. Draw a small volume of HBSS into the Pasteur pipet prior to picking the plaque to facilitate the expulsion of the plaque from the Pasteur pipet. Transfer the plaque material to a sterile capped tube containing 0.5 ml of 2 x Eagle's media. One plaque will be passed in SPAFAS eggs and the others should be frozen at -70°C as backup material; 4) pass one plaque in two SPAFAS eggs (0.2 ml of inoculum per egg) at 33°C for 72 hr. Refrigerate eggs at 4°C for at least one hr prior to harvesting the allantoic fluid. Determine the hemagglutinin titer(HA) of the egg pool to confirm the presence of virus and determine plaquing dilutions for the next purification. Two eggs will provide all the virus needed; and 5) genotype the 1PI egg material following the genotype procedure to identify potential 6/2 candidates.

**2PI (2nd) Plaque Purification.** Second plaque purification and genotype screening were performed as follows: 1) plaque the 1PI egg material in SPF-PCK cells at 33°C following the procedure for plaquing in PCK cells. Use the appropriate dilutions to obtain well-isolated plaques, such as the following:



TABLE 7

HA Titers	Approximate Dilutions
< 1:32	$10^{-3}$ and $10^{-4}$
$\leq$ 1:128	$10^{-4}$ and $10^{-5}$
$\leq$ 1:512	$10^{-5}$ and $10^{-6}$
> 1:512	$10^{-5}$ , $10^{-6}$ and $10^{-7}$

5

2PI plaques should be derived from the same material which is genotyped since the egg passage may exert selective pressure on the plaques; and 2) pick several  
10 plaques following the procedure previously described. One plaque will be replaques in SPF-PCK cells and the others should be frozen at -70°C as backup material.

**3PI (3rd) Plaque Purification.** Third plaque purification and genotype screening were performed as follows: 1) plaque the 2PI plaques in SPF-PCK cells at 33°C following the procedure for plaquing in PCK cells. The appropriate dilutions for  
15 this passage are  $10^{-1}$  and  $10^{-2}$ ; 2) pick several plaques following the procedure previously described. At this time you should know which are potential 6/2's and non-candidates can be discarded. One plaque will be amplified in SPAFAS eggs at 33°C and the others should be frozen at -70°C as backup material; 3) genotype the 6/2 candidates to confirm that 3PI passages have the 6/2 gene configuration; and 4)  
20 characterize the phenotypic profile of the 6/2 vaccine candidates at 25°C, 33°C and 37°C to confirm the presence of the *ca* and *ts* markers.

### C. INFLUENZA VIRUS

A number of cold-adapted reassortants and cold-adapted influenza vaccines (CAIV) have been produced and clinically tested using the general scheme set forth  
25 above with modifications known to or easily devisable by those skilled in the art without undue experimentation. In addition, the cold-adopted influenza vaccines that have proven efficacious are set forth in Table 10. The following Table sets forth the Type A and Type B reassortants:



TABLE 8

CAIV	
TYPE A REASSORTANT	TYPE B REASSORTANT
A/Victoria/75 (H3N2)	B/Tecumseh/63/80
A/Victoria/75 (H3N2)	B/Texas/1/84
A/Swine/New Jersey/8/76/ (H1N1)	B/Ann Arbor/1/86
A/Alaska/6/77 (H3N2)	B/Yamagata/16/88
A/Alaska/6/77 (H3N2)	B/Bangkok/163/90
A/USSR/90/77 (H1N1)	B/Panama/45/90
A/Hong Kong/77 (H1N1)	B/Panama/45/90
A/California/10/78 (H1N1)	
A/Alaska/6/77 (H3N2)	
A/Peking/2/79 (H3N2)	
A/Washington D.C./897/80 (H3N2)	
A/Shanghai/31/80 (H3N2)	
A/Korea/1/82 (H3N2)	
A/Dunedin/6/83 (H1N1)	
A/Bethesda/1/85 (H3N2)	
A/Texas/1/85 (H1N1)	
A/Kawasaki/9/86 (H1N1)	
A/Wyoming/1/87 (H3N2)	
A/Los Angeles/2/87 (H3N2)	
A/Shanghai/11/87 (H3N2)	
A/Shanghai/16/89 (H3N2)	
A/Guizhou/54/89 (H3N2)	
A/Chick/Germany/N/49 (H10N7)	
A/Equine/Miami/1/63 (H3N8)	
A/Beijing/352/89 (H3N2)	
A/Yamagata/32/89 (H1N1)	
A/Texas/36/91 (H1N1)	
A/Beijing/352/89 (H3N2)	
A/Los Angeles/2/87 (H3N2)	



## **SPECIFIC EXAMPLE 4 - CA INFLUENZA VIRUS REASSORTANT**

### **Vaccine Pools**

**Facilities.** The inoculation, harvesting, pooling, and filling operations were  
5 performed in a Biohazard Laminar Flow Hood (Type A/B3). All containers and  
equipment utilized were sterilized within 72 hr prior to use.

**Production Substrate.** Ten-day old incubated, specific pathogen free -  
complement fixation avian leukosis (SPF-COFAL) negative embryonated hens' eggs  
from SPAFAS, Inc. (Norwich, CT) were used. Quality Control Sheets for the flocks  
10 were obtained and retained to maintain traceability of eggs.

**Cold-adapted Reassortant Vaccine Donor Strain.** The cold-adapted  
reassortant vaccine donor strain passage will vary between SPF egg passage 1 (SE1)  
and SE4. These passages (SE1-SE4) of the donor virus were produced as follows:  
The virus was thawed and diluted 1:100 to 1:10,000 (strain-dependent) in HBSS.  
15 Ten-day old embryonated eggs were inoculated via the allantoic route with 0.1 ml of  
the indicated diluent. All eggs were incubated at 33°C for 40-72 hr (strain-dependent)  
at which time they were chilled at 4°C for 1-2 hr prior to the harvesting of the allantoic  
fluid. This material was passed once to prepare the seed lot.

#### **A. Virus Seed Production**

20 The virus seed lot was used as the seed for the production of all vaccine  
pools. All work was done in production facilities.

**Inoculation.** The seed virus was thawed and diluted 1:100 to 1:10,000 (strain-  
dependent) in HBSS containing 1% of 10 x SPG (sucrose, 2.18M;  $\text{KH}_2\text{PO}_4$ , 0.038M;  
 $\text{K}_2\text{HPO}_4$ , 0.072M; potassium glutamate, 0.049M). The ten-day old embryonated eggs  
25 were inoculated via the allantoic route with 0.1 ml of the indicated diluent. All eggs  
were incubated at 33°C for 40-72 hr (strain-dependent) at which time they were  
candled and any dead embryo was discarded. All live eggs were chilled overnight at  
4°C prior to the harvesting of the allantoic fluid.

**Harvest and Clarification.** Allantoic fluids were harvested and pooled in  
30 approximately 180 ml amounts. The harvested allantoic fluid was incubated at 37°C  
(water bath) for 60 min to elute any virus adsorbed to red blood cells. Each bottle  
was then clarified by centrifugation at 1400 g for 15 min. 10 x SPG was added to  
each harvest to achieve a 10% v/v suspension for virus stabilization. The harvest  
bottles were pooled. Sterility assays were carried out on the pool (dual sterility tests  
35 in both fluid thioglycollate and tryptone soya broth at 33°C and 22°C.). The seed pool  
was assayed for hemagglutinin activity and aliquotted in the appropriate volumes  
needed for vaccine production.



## **B. Virus Pool Production**

**Inoculation.** The seed virus was thawed and diluted 1:100 to 1:10,000 (strain-dependent) in HBSS containing 1% of 10 x SPG. The ten-day old embryonated eggs were inoculated via the allantoic route with 0.1 ml of the indicated diluent. For  
5 negative controls, approximately 30 eggs were inoculated via the allantoic route with 0.1 ml of the indicated diluent. All eggs were incubated at 33°C for 40-72 hr (strain-dependent) at which time they were candled and dead embryos were discarded. All live eggs were chilled overnight at 4°C prior to the harvesting of the allantoic fluid.

**Harvest and Clarification.** Allantoic fluids were harvested and pooled in  
10 approximately 180 ml amounts. The harvested allantoic fluid was incubated at 37°C (water bath) for 60 min to elute any virus adsorbed to red blood cells. Each bottle (control and infected) was then clarified by centrifugation at 1400 g for 15 min. 10 x SPG was added to each harvest to achieve a 10% v/v suspension for virus stabilization. Aliquots were removed from each harvest bottle to form a sample master  
15 pool. Sterility assays were carried out on each individual bottle and on the sample master pool; dual sterility tests in both fluid thioglycollate and tryptone soya broth at 33°C and 22°C were conducted. The master pool was assayed for hemagglutinin activity and virus characterization (phenotype and genotype assays).

**Pooling, Treatment and Dispensation.** When the preliminary tests (sterility  
20 and virus characterization) proved satisfactory, the sterile harvests were thawed and pooled. Fluids were passed through sterile gauze pads to remove any membranous material that may be present. Antibiotics were added to the final pools to achieve the following concentrations: neomycin 100 mcg/ml, amphotericin B (I.V.) 5 mcg/ml.

**Control Fluids:** This pool was distributed into the appropriate aliquots needed  
25 for subsequent testing for adventitious agents. During dispensation the fluid was kept chilled in an ice-water bath. The fluids were stored at < -75°C in a mechanical freezer.

**Virus-Infected Fluids:** This pool was distributed into the appropriate aliquots needed for subsequent safety testing. The remainder of the fluid was distributed into aliquots for use as a live cold-adapted influenza virus vaccine. During dispensation  
30 the fluid was kept chilled in an ice-water bath. The fluids were stored at < -75°C in a mechanical freezer.

**Tests for Adventitious Agents.** The following are microbial sterility tests: 1) pre-antibiotic testing for bacteria with fluid thioglycollate at 22°C and 33°C, and tryptone soya broth media at 22°C and 33°C; and 2) post-antibiotic testing for bacteria  
35 in Lowenstein-Jensen egg medium, and for mycoplasma and brucella.



Identity in tissue culture is tested using serum-neutralization in Primary African Green Monkey Kidney (AGMK) cells.

Tissue culture tests for adventitious agents are performed using : 1) Primary African Green Monkey Kidney (AGMK) cells; 2) Primary Bovine Embryonic Kidney (BEK) cells; 3) Primary Human Amnion (PHA) cells; 4) Primary Rabbit Kidney (PRK) cells; 5) Human Diploid Fibroblast (MRC-5) cells; and 6) Human Carcinoma of the Cervix (HeLa) cells.

Animal tests for adventitious agents are performed using: 1) adult mice (ICR); 2) suckling mice (CD-1); and 3) adult guinea pigs. Guinea pig tests are conducted for *M. tuberculosis*, Q-fever and *B. abortus* antibodies.

A test for reverse transcriptase by assaying for the detection of RNA-dependent DNA-polymerase activity is also performed.

Final container/pool testing is performed by the following tests: microbial sterility is tested with fluid thioglycollate at 22°C and 33°C and fluid soybean-casein digest; COFAL testing is performed to test for avian leukosis virus; general safety testing using mice and guinea pigs; virus characterization including infectivity with TCID<sub>50</sub> in Madin-Darby Canine Kidney (MDCK) cells, plaquing efficiency with Madin-Darby Canine Kidney (MDCK) cells with Plaque Forming Unit (PFU) determination at 34, 36, 37, 38 and 39°C, and SPF derived Primary Chick Kidney (SPCK) cells with Plaque Forming Unit (pfu) determination at 25, 33 and 39°C for confirmation of phenotypic markers; antigenic analyses using hemagglutinin inhibition assay and neuraminidase inhibition assay; reactogenicity in ferrets; hemagglutinin activity; and passage level, wherein the final passage of the vaccine will vary between SPF Egg Passage 3 (SE3) and SE6.

## **SPECIFIC EXAMPLE 5 - CHARACTERIZATION OF CA VACCINES**

### **A. CA VACCINE EVALUATION**

Production lots of cold-adapted influenza vaccines were evaluated prior to distribution to certify that they were identical to the seed strains from which they were produced. The production lots underwent three different tests to certify that they were identical to the seed strains: phenotypic evaluation, genotypic evaluation and ferret reactogenicity studies.

**Phenotypic Evaluation of Cold-adapted Influenza Vaccines.** Cold-adapted influenza vaccines contain two stable phenotypic markers, the cold-adapted (*ca*) marker and the temperature-sensitive (*ts*) marker. Presence of the *ca* marker is confirmed by comparable viral growth at 25°C and 33°C. The *ts* marker is confirmed by a minimum 100-fold decrease in viral growth at 39°C as compared to 33°C for the



Type A cold-adapted influenza vaccine. Viral growth is quantified as plaque-forming units/milliliter (pfu/ml) in primary chick kidney cells. Production lots are checked to certify that they have both of the phenotypic markers.

**Genotypic Evaluation of Cold-adapted Influenza Vaccines.** Influenza viruses  
5 are negative-stranded RNA viruses with eight unique strands of RNA, each of which corresponds to an individual gene. As described above, the cold-adapted influenza vaccine is a 6/2 reassortant which contains the six attenuated internal genes of the master strain parent with the two genes coding for the surface antigens of the wild type parent. Since the genes have different electrophoretic mobilities, they can be  
10 differentiated via polyacrylamide gel electrophoresis. Production lots are checked to certify that they have the 6/2 gene constellation of the seed strain.

**Ferret Reactogenicity Studies.** The ferret is the animal model of choice for testing the potential virulence of influenza viruses. The cold-adapted influenza vaccine is attenuated in ferrets and is characterized by an asymptomatic infection with viral  
15 growth restricted to the nasal turbinates. In this study, a ferret was infected with a high multiplicity of infection dose and monitored twice daily for symptoms of influenza. On day 3, the peak day for viral replication, the ferret was euthanized and the turbinate and lung were checked for viral growth. Production lots were checked to confirm that they are attenuated in the ferret model.

## 20 B. MATERIALS AND METHODS

### Preparation of PCK Cells

**Media and Materials.** The media used in this example are prepared with the following components: a) 199 with 10% FBS - 450 ml sterile Type I deionized water; 50 ml Fetal Bovine Sera - heat inactivated; 50 ml 10 x 199 (GIBCO #330-1181); 10  
25 ml L-glutamine (GIBCO 320-5030); 0.5 ml gentamicin sulfate (50mg/ml) (M.A. Bioproducts 17-518); and 16 ml 1.4% NaHCO<sub>3</sub>, pH to 6.8 with 0.5N NaOH. HBSS w/P&S - 500 ml HBSS (M.A. Bioproducts 10-508); and 0.5 ml gentamicin sulfate (50mg/ml) (M.A. Bioproducts 17-518); b) 0.25% trypsin - 1 L HBSS (M.A. Bioproducts 10-508); and 2.5 g trypsin 1:250 (Difco 0152-15-9). Dissolve in HBSS by stirring at  
30 room temperature, filter sterilize (0.22 $\mu$ ), pH to 7.6 with 0.5N NaOH after filtering; c) 0.5N NaOH - 2 g NaOH; and 100 ml Type I deionized water; sterilize by autoclaving 250°C for 15 min, liquid cycle; d) 1.4% NaHCO<sub>3</sub> - 100 ml Type I deionized water; 1.4 g NaHCO<sub>3</sub>; and 0.1 ml 4% Phenol Red. Sterilize - autoclave 250°C for 15 min, liquid cycle; e) 4% Phenol Red - 2 g Phenol Red (Difco 0203-11-2); 39 ml Type I deionized  
35 water; and 11 ml 0.5N NaOH; sterilize by autoclaving 250°C for 15 min, liquid cycle.



The following materials are also used: sterile instruments; sterile cotton balls; sterile gauze; sterile Petri dish; sterile 50 ml centrifuge tubes; ether jar and diethyl ether; dissecting boards and pins; and 70% ethanol.

**Procedure.** The following procedures are performed: 1) sacrifice 1 to 3-day  
5 old chicks with ether; 2) place chicks on dissecting board (backs against board) and pin the wings and feet; 3) wash chick with 70% ethanol; 4) cut away skin starting at throat to totally expose chest and abdomen using one set of sterile instruments; 5) using a second set of sterile instruments, cut along each side of the rib cage, peel down rib cage and omentum to expose internal organs; 6) with new sterile instruments  
10 cut the esophagus and trachea, peel down internal organs to expose the kidneys; 7) swab the body cavity with sterile cotton balls to remove blood; 8) with new sterile instruments remove kidneys and place in a Petri dish with HBSS; 9) with new sterile instruments remove connective tissue from the kidneys; 10) transfer kidneys to a 50 ml centrifuge tube. Keep the kidneys near the top for mincing; 11) mince the kidneys  
15 with a new set of instruments, using recurved scissors; 12) wash the kidneys three times with HBSS (10 ml per wash) and discard all washes; 13) add 5 ml of 0.25% trypsin per chick and incubate at 35°C for ten min with occasional shaking; 14) shake vigorously by hand for three minutes. (The trypsinization times can vary with the activity of each lot of trypsin used); 15) centrifuge for 10 min at 1000-1200 RPM; 16)  
20 pour off supernatant and resuspend cells in 10 ml of 199 w/ 10% FBS; 17) filter through sterile gauze into 20 ml per chick of 199 w/ 10% FBS, and dispense into culture flasks, tubes or plates and incubate at 35°C; 18) feed 100% with 199 w/ 10% FBS after 72 hr; and 19) incubate at 35°C, cells should be usable 96 hr after seeding.

#### **Plaquing PCK Cells**

25 **Media.** The following media are used: a) Kilbourne - 350 ml sterile Type I deionized water; 100 ml 10 X 199 (GIBCO #330-1181); 20 ml MEM amino acids (50X) (M.A. Bioproducts 13-606); 7.5 ml 5% NaHCO<sub>3</sub>; 10 ml MEM vitamins (100X) (M.A. Bioproducts 13-607); 2.86 ml 35% Bovine Sera Albumin (SIGMA A-8918); and 0.5 ml gentamicin sulfate (50mg/ml) (M.A. Bioproducts 17-518) adjust pH to 7.0 using 0.5N  
30 NaOH; b) 2 x Eagle's - 500 ml HBSS (M.A. Bioproducts 10-508); 10 ml BME amino acids (GIBCO 320-1051); 10 ml BME vitamins (GIBCO 320-1040); 10 ml L-glutamine (GIBCO 320-5030); and 0.5 ml gentamicin sulfate 50 mg/ml (M.A. Bioproducts 17-518) adjust pH to 7.0 using 0.5N NaOH; c) 1% DEAE dextran - 1 g DEAE dextran (Pharmacia 17-0350-01); and 100 ml sterile Type I deionized water (Filter Sterilize  
35 (0.22 $\mu$  filter)); d) 1% Neutral Red - 1 g Neutral Red (DIFCO Bacto Neutral Red 0208-13); 100 ml sterile Type I deionized water; 1) dissolve in H<sub>2</sub>O by stirring at room



temperature for several hours; 2) filter through Whatman #1 filter paper to remove undissolved particulates; 3) aliquot into light-proof bottles and autoclave to sterilize (15 psi for 15min); and 4) store at room temperature (works best when the stain has aged; unlimited shelf life); e) HBSS - 500 ml HBSS (M.A. Bioproducts 10-508); and 0.5 ml gentamicin sulfate 50 mg/ml (M.A. Bioproducts 17-518), adjust pH to 7.0 using 0.5N NaOH; f) 0.5N NaOH - 2 g NaOH; and 100 ml Type I deionized water. Sterilize by autoclaving 250°C for 15 min, liquid cycle; g) 1.6% purified agar - 1.6 g BBL agar purified (Becton Dickison 11853); and 100 ml sterile Type I deionized water. Autoclave to sterilize and prepare while virus is adsorbing - make volume needed for overlay.

10       **Procedure.** The following procedure was used: 1) set up water bath to keep media and agar at proper temperature (39-41°C); 2) make serial dilutions of the virus in 2 x Eagle's (1 ml of diluted virus per 25 cm<sup>2</sup> tissue culture flask); 3) remove media from tissue culture flasks and wash once with HBSS, 2 ml per 25 cm<sup>2</sup> flask; 4) add 1 ml of diluted virus per 25 cm<sup>2</sup> flask; 5) adsorb virus at room temperature for 1 hr with gentle rocking; 6) remove virus inoculum from flask; 7) overlay with a 1:1 mixture as described below, 5 ml per 25 cm<sup>2</sup> flask (1st Overlay - see below); 8) cool bottles until agar gels at room temperature, approximately 10 min; 9) incubate at desired temperatures (Type A Influenza - Phenotype 25°, 33° and 39°C; Type B Influenza - Phenotype 25°, 33° and 37°C); 10) after appropriate incubation overlay with 1:1 mixture as described below, 4 ml per 25 cm<sup>2</sup> flask (2nd Overlay - see below);

TABLE 9

Temperature	Incubation until 2nd overlay
25°C	96 hr
33°,37°,39°C	48 hr

25       11) cool bottles until agar gels at room temperature, approximately 10 min; 12) incubate at desired temperature; and 13) check daily for plaques. At 33°, 37° and 39°C, all plaques should be visible within 48 hr after the second overlay. At 25°C, it can take up to 168 hr (7 days) after the second overlay for all plaques to be visible.

30       The 1st Overlay is prepared by a 1:1 mixture of the following media mixture with 1.6% purified agar: 100 ml Kilbourne media and 3 ml 1% DEAE dextran. The amount of DEAE dextran needed will vary with the batch of purified agar. This concentration should work for most batches.

35       The 2nd Overlay - Neutral Red is prepared by a 1:1 mixture of the following media mixture with 1.6% purified agar: 100 ml Kilbourne media; 3 ml 1% DEAE dextran. The amount of DEAE dextran needed will vary with the batch of purified agar.



This concentration should work for most batches; and 2 ml 1% Neutral Red. The amount of Neutral Red needed can vary with the batch. For long-term consistency, enough Neutral Red can be made at one time to last several years.

### RNA Labelling

- 5        **Media and Solutions.** The following media and solutions are used: a) HBSS - 500 ml HBSS (M.A. Bioproducts 10-508); and 0.5 ml gentamicin sulfate 50 mg/ml (M.A. Bioproducts 17-518) (adjust pH to 7.0 using 0.5N NaOH); b) 2 x Eagle's -500 ml HBSS (M.A. Bioproducts 10-508); 10 ml BME amino acids (GIBCO 320-1051); 10 ml BME vitamins (GIBCO 320-1040); 10 ml L-glutamine (GIBCO 320-5030); 0.5 ml
- 10 gentamicin sulfate 50 mg/ml (M.A. Bioproducts 17-518) (adjust pH to 7.0 using 0.5N NaOH); c)  $^3\text{H}$ -uridine - [5,6- $^3\text{H}$ ] Uridine - 1.0 mCi/ml (Amersham, Inc. TRK 410); d) 5 M NaCl - 146.1 g NaCl (Bring the volume to 500 ml with Type I deionized water); e) 1 M Tris-HCl (pH 7.4) - 60.55 g Trizma Base (Sigma T-1503); 400 ml Type I deionized water; 35 ml concentrated HCl; and 0.5 ml diethylpyrocarbonate (Sigma D-5758);
- 15 Allow solution to cool to room temperature. Adjust pH to 7.4 with HCl. Bring the volume up to 500 ml with Type I deionized water. Sterilize by autoclaving 250°C for 15 min, liquid cycle; e) 0.5 M EDTA - 186.1 g disodium EDTA (Sigma ED2SS); 800 ml Type I deionized water; and 20 g NaOH. Mix and adjust the pH to 7.4 with NaOH, sterilize by autoclaving 250°C for 15 min, liquid cycle; f) 30% sucrose - 150 g sucrose
- 20 (Sigma S-9378); 10 ml 5 M NaCl; 5 ml 1 M Tris-HCl, pH 7.4; and 1 ml 0.5 M disodium EDTA (ethylenediaminetetraacetic acid) (Sigma ED2SS). Bring up to 500 ml with Type I deionized water; g) STE (Sodium-Tris-EDTA) - 1 ml 0.5 M disodium EDTA (Sigma ED2SS); 10 ml 5.0 M NaCl; and 5 ml of 1 M Tris-HCl, pH 7.4 (Trizma Base) (Sigma T-1503). Add 484 ml of Type I deionized water; h) proteinase-K - proteinase-K 20
- 25 mg/ml (Beckman-340321). Dilute 100 mg in 5 ml of sterile Type I deionized water; i) SDS - sodium dodecyl sulfate (Sigma L-5750), 10% (w/v) in Type I deionized water; j) 1/10 x TBE loading buffer - 0.5 ml 10 x TBE; 0.5 ml 10% SDS; 1.0 g ficoll (Sigma F-4375); 2.5 ml glycerol (Baker 2140-03); and 0.125 g Bromophenol Blue (Bio-Rad 161-0404). Bring up to 50 ml with Type I deionized water.

- 30        **Protocol.** The following protocol is used:

- Day 1: 1) Use 2 - 25 cm<sup>2</sup> flasks of primary chick kidney cells; 2) remove media and wash with HBSS, 2 ml/flask; 3) infect cells with virus - 2 ml virus diluted 1:2 in 2 x Eagle's; 4) rock cells gently for 1 hr at room temperature; 5) remove inoculum; 6) add label, use 0.2 mCi - 0.25 mCi  $^3\text{H}$ -uridine/flask. Diluted in 2 x Eagle's, total volume
- 35 1.5 ml/flask; 7) place in 33°C incubator for 4 hr; 8) after 4 hr, add 3.5 ml 2 x Eagle's to each flask; and 9) incubate at 33°C for 48 hr.



Day 3: 1) Transfer fluid from the 2 flasks into a 15 ml centrifuge tube; 2) centrifuge at 500 g for 15 min at 4°C; 3) pour supernatant into 30 ml <sup>OAKRIDGE test tube</sup> Oakridge tubes; 4) underlay supernate with 7.5 ml 30% sucrose; 5) balance tubes with STE; 6) spin at 22,500 rpm for 2-1/2 hr in a Beckman type 30 rotor; 7) pour fluid from tubes into beaker (<sup>3</sup>H aqueous waste - discard); 8) let tubes sit on paper inverted for 5-10 min; 9) mark pellet - dry tube with <sup>PAPER (e.g., KIMWIPES)</sup> Kimwipe; 10) resuspend each pellet in 200 µl STE, place suspension in a 1.5 ml centrifuge tube; 11) add 8 µl proteinase K (0.16 mg) to each tube, mix and incubate at 37°C for 10 min; 12) add 10 µl of 10% SDS. Mix and incubate at 37°C for 10 min; and 13) add 0.65 ml of 95% EtOH. Mix and place at -20°C overnight.

Day 4: 1) Pellet the RNA in a microcentrifuge for 15 min at 4°C; 2) empty EtOH into beaker - drain tubes upside down for several min; 3) dry the tubes in a <sup>SPEED VAC - TYPE</sup> Speedvac concentrator for approximately 10-20 min; 4) resuspend pellet in 32 µl of 1/10 x TBE loading buffer; 5) heat at 56°C for 2-3 min; 6) remove 2 µl sample and mix with 2 ml of liquid scintillation fluid; 7) count on Channel 1 for 0.5 min in liquid scintillation counter to get CPM (counts per min); 8) freeze sample until used at -70°C; 9) heat at 56°C for 2-3 min before loading; and 10) load 150,000 - 200,000 CPM.

#### Mixed Agarose-PAGE

**Reagents.** The following reagents were employed: a) 30% acrylamide, 1.5% bis-acrylamide - 30 g acrylamide (Bio-Rad 115009B); and 1.5 g bis-acrylamide (Bio-Rad 41936B). Bring up to 100 ml with Type I deionized water; b) 10 x TBE Buffer - 54 g Trizma Base (0.89 M) (Sigma T-1503); 27.5 g boric acid (0.89 M) (Mallinckrodt CAS10043-35-3); 4.65 g EDTA disodium salt (20 mM); (ethylenediaminetetraacetic acid) (Sigma ED2SS). Bring up to 500 ml with Type I deionized water; c) 10% w/v SDS - 10 g sodium dodecyl sulfate (Sigma L-5750). Bring up to 100 ml with Type I deionized water; d) diethylpyrocarbonate - diethyl pyrocarbonate 50 ml in 100 ml deionized water (Sigma D-5758); e) 1 x TBE running buffer - 216 g Trizma Base (89 mM) (Sigma T-1503); 110g boric acid (0.89 M) (Mallinckrodt CAS10043-35-3); 18.6 g EDTA disodium salt (20 mM) (Sigma ED2SS); (ethylenediaminetetraacetic acid); and 20 g sodium dodecyl sulfate (SDS) (0.1%) (Sigma L-5750). Bring up to 20 liters with Type I deionized water and mix well; f) 10% ammonium persulfate - 0.3 g ammonium persulfate (Bio-Rad M3992); bring up to 3.0 ml. Stable for 7 days at 4°C; g) TEMED - tetramethylethylenediamine (Bio-Rad 161-0801); h) agarose - Type V - high gelling temperature (SIGMA A-3768); i) salicylic acid - 0.3 g salicylic acid (Sigma S-3007); 36 g hexadecyltrimethylammonium bromide (Sigma H-5882); and 300 ml Type I deionized water.



**Procedure.** The following procedure is used for mixed acrylamide/agarose gel (3.0% acrylamide/0.6% agarose): Note that for proper polymerization of the gel, it must be at 56°C for 20 min after pouring. The standard procedure is to place the plates vertically in a 56°C water bath such that the water is within 1 inch of the plate tops 1) Combine and mix for 15 min: 0.6 g agarose Type V high gelling temperature, 92 ml Type I deionized water, and 50 µl diethylpyrocarbonate; 2) boil until volume is below 79 ml; 3) measure in graduated cylinder, bring volume to 79 ml with sterile Type I deionized water, allow to cool slightly; 4) add: 10 ml of 10 x TBE, 10 ml of 30% Acrylamide/1.5% bis acrylamide, 1 ml of 10% SDS, 0.3 ml of 10% ammonium persulfate; and 30 µl TEMED; and 5) gently mix and pour the gel immediately. After the gels have polymerized (20 min at 56°C), they are stored overnight in running buffer prior to use.

The gels are run at a constant temperature in a circulating buffer system. Since the gels are run for extended periods (17 to 21 hr) the circulation of the running buffer is critical. The gels are run at temperatures ranging from 26°C to 40°C, and at either 230 or 240 volts (constant voltage) for 17 to 24 hr. The following are general guidelines for genotyping cold-adapted influenza vaccines: Type A: 30°C and 37°C (two gels run) at 230 volts for 17 hr. Type B: 26°C and 36°C (two gels run) at 240 volts for 21 hr.

After gels are run they are enhanced in salicylic acid for 45 min and then dried. The dried gels are placed in cassettes with X-ray film at -70°C and exposed for 24 to 72 hr. The film is developed and genotypes are read.

#### **Ferret Reactogenicity Testing**

**Media and Materials.** The following media and materials are used: a) 2 x Eagle's - 500 ml HBSS (M.A. Bioproducts 10-508); 10 ml BME amino acids (GIBCO 320-1051); 10 ml BME vitamins (GIBCO 320-1040); 10 ml L-glutamine (GIBCO 320-5030); and 0.5 ml gentamicin sulfate 50 mg/ml (M.A. Bioproducts 17-518) adjust pH to 7.0 using 0.5N NaOH. b) sodium pentobarbital - sodium pentobarbital injection (65 mg/ml) Anthony Products Co.; c) alundum - 60 mesh norton alundum "RR" (Fisher Scientific Co. A-620); sterilize by autoclaving at 250°C for 15 min, dry cycle. Ferrets - 8 to 10-week old ferrets, male, castrated, and vaccinated against distemper (Marshall Research Animals). If the ferrets are not barrier-raised, they may have had an influenza infection during the influenza season. The animals will thus need to be treated with Penicillin G (30,000 units/day) for 7 days prior to use. (Durapen TM combination antibiotic (Vedco); and Penicillin G Benzathine and Penicillin G Procaine, 300,000 units/ml.) Miscellaneous - sterile instruments; sterile scalpel; diethyl ether for



*Lysol-type disinfectant* - 36 -

anesthesia; *lysol*, sterile Petri dishes; sterile mortar and pestle; and digital thermometer Model 8110-20 (Cole Parmer Instrument Company).

**Protocol.** The following protocol is used:

- Day 1: 1) Dilute the stock virus  $10^{-1}$  in 2 x Eagle's; 2) lightly anesthetize the ferret with diethyl ether. Inoculate ferret intranasally with 1 ml of the  $10^{-1}$  dilution of stock virus (0.5 ml in each nostril); 3) determine the  $EID_{50}/ml$  (Egg Infectious Dose-50%/ml) titer of the inoculum; serially dilute the inoculum in 2 x Eagle's; inoculate 9-11 day old embryonated chicken eggs with dilutions  $10^{-5}$  through  $10^{-8}$ , four eggs per dilution (0.1 ml per egg); incubate the eggs at  $33^{\circ}C$  to  $35^{\circ}C$  for 72 hr; after 72 hr cool the eggs for several hr at  $4^{\circ}C$ ; remove 1 ml of allantoic fluid from each egg and place in individual Kahn tubes; add 0.5 ml of 0.5% chicken red blood cells to each tube and mix; allow the blood to precipitate for 45 min and determine which tubes are positive for hemagglutinin activity. Calculate the  $EID_{50}$  titer using the Reed-Meunch method; and 4) take rectal temperatures twice a day for 3 days.
- Day 3: 1) The ferret is euthanized via heart puncture with sodium pentobarbital (130 mg/ferret); 2) place ferret on its back and clamp feet to immobilize; 3) wash abdomen with *Lysol*®; 4) using sterile forceps and scalpel make a 4-5 inch incision lengthwise down the sternum and pull skin back; 5) with new set of sterile forceps and scissors cut the ribs to make an opening large enough to remove the left lower lobe of lung; remove and place in a sterile Petri dish; 6) cut a section of the left lobe into small pieces and place into a freezable storage tube; 7) turn ferret over and wash head with *Lysol*®; 8) with scalpel and forceps remove the skin from the end of nose to below eyes; 9) cut off snout at the base of the septum; 10) cut the nasal bone on both sides of the septum - approximately  $1/8$  to  $1/4$  inch with sterile bone cutter; 11) scrape out the turbinate using sterile curette and place in freezable storage tube; 12) weigh the tubes containing the lung and turbinate samples and record; 13) place the tissues in sterile mortars and weigh the empty tube. The difference in the weight is the weight of tissues; 14) add sterile alundum to the mortars and grind (homogenize) the tissues with a sterile pestle; 15) dilute tissue with 2 x Eagle's to make 10% weight/volume suspension; 16) centrifuge the homogenate at  $500 \times g$  for 10 min at  $4^{\circ}C$ ; 17) remove supernatant and freeze at  $-70^{\circ}C$ ; 18) thaw the supernatant and determine the  $EID_{50}/ml$  as previously described. A general range for inoculation is: 3-day turbinate dilutions of  $10^{-3}$  to  $10^{-6}$  dilution, 3-day lung dilutions of  $10^{-1}$  to  $10^{-4}$  dilution; and 19) harvest the eggs from the inoculum and calculate the  $EID_{50}$  as described previously.



Day 6: 1) Harvest the eggs from the 3-day turbinate and lung and calculate the  $EID_{50}$ 's as described previously.

#### Ferret Serum Collection

**Materials.** The following materials are used: B-D <sup>VACUTAINER</sup> brand Winged  
5 Collection Set, 19 gauge needle, with luer; adapter and 12-inch tubing (B-D #4919);  
B-D Vacutainer brand needle holder for 16mm tube (B-D #364888); B-D Vacutainer  
brand SST (Serum Separation Tube) 16 x 125mm (B-D #6512); diethyl ether for  
anesthesia; and 70% ethanol.

**Procedure.** The following protocol is employed: 1) assemble collection set  
10 and needle holder; 2) lightly anesthetize the ferret with diethyl ether; 3) place ferret on  
its back and hold firmly; 4) wash chest with 70% ethanol; 5) palpate for heartbeat (left  
side, between 3rd and 4th rib from base of sternum; 6) insert needle into ferret's heart;  
when blood is seen entering the collection tube insert the SST tube onto needle;  
collect the desired amount for test procedures; 3-4 ml of blood will provide 1-2 ml of  
15 serum; 7) allow blood to completely clot at room temperature (approx. 30 min); 8)  
centrifuge tube at room temperature for 10 min at 1000 - 1300 g; and 9) collect serum,  
aliquot, and store at -70°C. Note that ferret serum should be treated using the  
trypsin-periodate method described below to remove nonspecific inhibitors prior to  
use.

#### 20 Trypsin-Periodate Treatment for Ferret Sera

**Materials.** The following materials are used: a) phosphate buffer for trypsin;  
Solution A consists of  $NaH_2PO_4 \cdot H_2O$  (MW 138.01); 6.99 g  $NaH_2PO_4 \cdot H_2O$ ; and 500 ml  
sterile Type I deionized water. Solution B consists of  $Na_2HPO_4$  (MW 141.97); 7.1 g  
 $Na_2HPO_4$ ; and 500 ml sterile Type I deionized water; b) working buffer consists of 1  
25 volume of Solution A + 31 volumes of Solution B (pH=8.2); c) Trypsin solution - 0.4g  
trypsin 1:250 (DIFCO 0152-13-1); and 100 ml phosphate buffer. Solution is stable  
when frozen at -20°C; d) potassium periodate solution - 0.255 g  $KIO_4$  (MW 230.02);  
and 100 ml sterile Type I deionized water. Store in a light-proof bottle. Stable at room  
temperature for one month; e) 1% glycerol saline - 1 ml glycerol; and 99 ml phosphate  
30 buffered saline (PBS) (M.A. Bioproducts 17-516).

Sera Treatment - 1) mix 1 volume of serum + 1 volume of trypsin solution; 2)  
heat immediately to 56°C for 30 min; 3) cool to room temperature; 4) add 3 volumes  
of potassium periodate solution; 5) mix and incubate at room temperature for 15 min;  
and 6) add 3 volumes of 1% glycerol saline; serum is a 1:8 dilution and is ready to  
35 use for HI tests. If serum is going to be used for making reassortants it needs to be  
filter sterilized through a 0.22 $\mu$  filter (low protein binding).



### **Hemagglutinin Inhibition Screening of Ferret Sera**

**Procedure.** The ferrets are screened prior to use to certify that they are immunologically naive to influenza virus. Follow the hemagglutinin inhibition procedure as described in: "Concepts and Procedures for Laboratory-Based Influenza Surveillance", U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control (July 1982).

Ferrets are screened for exposure to influenza strains which have circulated in the last 12 months and/or strains which are presently circulating. The ferret sera should always be screened against a Type A H1N1 strain, a Type A H3N2 strain, and the most recent Type B strain.

### **SPECIFIC EXAMPLE 6 - CLINICAL RESULTS**

Since 1976 the clinical development of the cold-adapted influenza virus vaccines has included the testing of multiple reassortant vaccines in over 20,000 people between the ages of 4 months to over 80 years. A summary of the cold-adapted influenza vaccines tested in the United States is set forth in Table 10. These studies have consistently demonstrated the ca vaccines to be genetically stable, and non-transmissible in all populations tested. More recently, studies on the ca vaccine have focused in three broad areas: 1) evaluating the range and extent of the immunologic response; 2) determining the protective efficacy of the vaccine in the overall population as well as in targeted subsets; and 3) evaluating the immunologic and efficacious consequences of administering divalent/trivalent ca influenza virus vaccines.

The following is a standard procedure for the clinical evaluation of and collection of specimens from volunteers in attenuated influenza vaccine studies.

#### **A. Clinical Observations**

Two observers should independently evaluate the clinical status of the volunteer. Optimally, each evaluator should see the patient daily before and during the first four days after virus administration.

**Categories of Illness.** 1) Fever - Oral temperature of greater than 37.7°C (100°F) will be considered a febrile reaction. Any temperature should be confirmed using a second thermometer, 5 minutes after the first measurement. If positive, measurement should be repeated every four hours. 2) Systemic Illness - Occurrence of myalgias, and/or chills and sweats are required for the assignment of systemic illness to a volunteer. Additional information should be gathered concerning feverishness, malaise, headache, anorexia, etc. It is recognized that these observations are subjective. 3) Pharyngitis - Sore, painful throat observed in 2



consecutive days. All volunteers reporting this symptom should receive appropriate bacterial diagnostic workups. 4) Rhinitis - Occurrence of rhinorrhea on two consecutive days. Presence of nasal obstruction and sneezing are supporting of this illness designation. 5) Lower Respiratory Tract Illness - A symptom complex  
5 consisting of substernal pain, cough (paroxysmal), sputum production.

**Administration of Virus to Volunteers.** An appropriate therapeutic dose, *i.e.* 0.25 ml, is administered to each nostril of a supine volunteer who should remain supine for at least ten minutes. Preferably the vaccine should be administered to all volunteers by the same individual.

10 **B. Clinical Specimens**

1) For virus isolation, nasal wash (NW) consisting of 5 ml of veal infusion broth, containing no antibiotics, is administered to each nostril. 0.25 ml of this wash should be inoculated into each of 4 tubes of an appropriate tissue culture (RMK or MDCK). The remaining NW should be divided into three aliquots and stored at -70C. 2) At  
15 least 20 ml of blood should be collected before immunization and at 21 to 28 days after immunization. An alternative method is the use of a nasopharyngeal swab and 2 ml of veal infusion broth with antibiotics for viral isolation. 3) Nasal wash for local antibody determination - 5 ml of a physiologic salt solution is instilled into each nostril and collected. A second specimen is collected at least 30 minutes later. These two  
20 collections are pooled. The timing of the pre- and post- immunization collections is the same as for serum. The specimens should be concentrated approximately 10 fold.

**C. Determination of Serum and Nasal Wash Antibody Levels**

The tests and antigens for screening the volunteers and evaluating serum and  
25 nasal wash antibodies is as follows: Screening of volunteers - All volunteers should be HI and NI negative to the influenza subtypes that are being evaluated in the study. The antigens to be used are the A/Denver/57 and A/USSR/90/77 (Parke Davis vaccine). NI antibody determinations are performed on the specimens.



**TABLE 10**  
**Summary of Cold-adapted (ca) Influenza Vaccines**  
**Tested In the United States**

ca Vaccine		Results			
		Attenuated	Antigenic	Genetic Stability	Efficacy
B/Hong Kong/73, CR-7	Adults	+	+	+	+
	Children	ND	ND	ND	ND
A/Victoria/75, (H3N2) CR-22	Adults	+	+	+	+
	Children	+	+	+	+
A/Alaska/77, (H3N2) CR-29	Adults	+	+	+	+
	Children	+	+	+	+
A/Hong Kong/77, (H1N1) CR-35	Adults	+	+	+	±
	Children	+	+	+	±
A/California/78, (H1N1) CR-37	Adults	+	+	+	+
	Children	+	+	+	+
A/Washington/80, (H3N2) CR-48	Adults	+	+	+	+
	Children	+	+	+	+
A/Korea/82, (H3N2) CR-59	Adults	+	+	+	±
	Children	+	+	+	±
A/Dunedin/83, (H1N1) CR-64	Adults	+	+	+	±
	Children	+	+	+	ND
B/Texas/84, CRB-87	Adults	+	+	+	+
	Children	+	+	+	ND
A/Bethesda/85, (H3N2) CR-90	Adults	+	+	+	+
	Children	+	+	+	+



ca Vaccine		Results			
		Attenuated	Antigenic	Genetic Stability	Efficacy
A/Texas/85, (H1N1) CR-98	Adults	+	+	+	+
	Children	+	+	+	+
A/Kawasaki/86, (H1N1) CR-125	Adults	+	+	+	+
	Children	+	+	+	+
B/Ann Arbor/86, CRB-117	Adults	+	+	+	ND
	Children	+	+	+	ND
A/Los Angeles/87, (H3N2) CR-149	Adults	+	+	+	+
	Children	+	+	+	+
B/Yamagata/88	Adults	+	+	+	ND
	Children	ND	ND	ND	ND

ND = not done



## SPECIFIC EXAMPLE 7 -

### SIMULTANEOUS ADMINISTRATION WITH OTHER VACCINES

One of the pressing needs for the development of the *ca* vaccine is to determine if protective immunogenicity is compromised when a bivalent or trivalent preparation is administered, and if so, if this interference can be overcome. Previous studies comparing monovalent and bivalent *ca* A vaccine (H1N1 and H3N2) administration in seronegative children demonstrated that the frequency of seroconversion was higher when vaccines were administered individually rather than simultaneously. Wright, P.F. et al., *J. Infect. Dis.* 146:71-79 (1982); Wright, P.F. et al., *Vaccine* 3:305-308 (1985). Using simultaneous administration of  $10^5$  tissue culture infectious doses (TCID<sub>50</sub>) of each of three *ca* vaccines (H1N1, H3N2 and B), (less than 10 human infectious doses {HID<sub>50</sub>}/vaccine component) Belshe and coworkers evaluated the question of trivalent vaccine interference in infants. Belshe, R.B. et al., *J. Infect. Dis.* 165:727-732 (1992). Among the seropositive children, few children shed vaccine virus and few increases in antibody to any of the three vaccine components was observed. Within the triply seronegative infant group, 47% shed all three *ca* vaccine viruses and 75% of these infants had a significant antibody rise to all three *ca* vaccine components. Of those that showed either shedding or antibody rise to two of the three *ca* vaccine components, no strain pair preference was observed. These results suggest that in infants and children not previously exposed to influenza, it may be possible to identify an appropriate dose (e.g. 100 HID<sub>50</sub>/vaccine component) which could stimulate antibody response to all three components.

The question of serological and/or protective interference in the adult population has been raised in relationship to the bivalent *ca* A vaccine efficacy studies. Edwards, K.M. et al. "A Randomized Controlled Trial of Cold-Adapted and Inactivated Vaccines for the Prevention of Influenza A Disease" (submitted for publication); Clover, R.D. et al., *J. Infect. Dis.* 163:300-304 (1991). Trivalent vaccine administration has recently been evaluated in adults having low antibody levels to all three components. In the adult population significant interference with virus shedding and a trend toward lower antibody responses, particularly against the *ca* B vaccine component, was observed in vaccinees receiving the trivalent *ca* vaccine when compared to either a bivalent A or monovalent B controls. Keitel, W.A. et al., "Trivalent Live Cold-adapted Influenza Virus Vaccine: Evidence for Virus Interference in Susceptible Adults." Manuscript in preparation). These results suggest that appropriate formulation may need to be developed to enhance the maximal response



of each influenza vaccine component. Thus, the present invention contemplates the use of such appropriate formulations which may be made by those skilled in the art.

#### **SPECIFIC EXAMPLE 8 - OTHER GENETICALLY-ENGINEERED VACCINES**

More recent techniques, such as recombinant DNA cloning and the  
5 transfection of *in vitro* mutagenized gene segments can be employed for the production of live virus vaccines. For example, the gene coding for the HA protein has been cloned into vaccinia virus and is expressed on the virus surface. Attenuated recombinant vaccinia viruses have been shown to provide protection to homologous *wt* virus challenge in hamsters. Smith, G.L. et al., *PNAS (USA)* 80:7155-7159 (1983).  
10 If necessary, other influenza genes cloned into the vaccinia virus carrier are also employed at the same time. Alternatively, master strains are comprised of a number of selected genes with specific mutations, including deletions to confer stability. Chanock, R.M. et al., *Prospects for Stabilization of Attenuation* in "The Molecular Virology and Epidemiology of Influenza", Stuart-Harris et al. (eds.) Academic Press, NY  
15 (1984). CR43-3 virus is a cold reassortant whose genome contains an NS gene with a deletion in the NS1 protein coding region and is restricted for growth in both Madin-Darby canine kidney cells and in ferrets. Buonagurio, D.A. et al., *J. Virol.* 49:418-425 (1984); Maassab, H.F. et al., *Virology* 130:342-350 (1983). Because the remaining non-(HA and NA) genes are derived from the *ca* master strain A/Ann Arbor/6/60 virus,  
20 CR43-3 may have the potential to be used as a new master strain.

Deletions are also generated through site specific mutagenesis in recombinant cDNA clones. The ability to introduce RNA transcripts of specifically mutagenized cDNA clones into the influenza viruses as stable parts of the genome has opened new areas of research into vaccine development. Enami, M. et al., *J. Virol.* 65:2711-2713  
25 (1991); Enami, M. et al., *PNAS (USA)* 87:3802-3805 (1990). It is now thus possible to produce "tailor-made" influenza vaccines engineered for specific purposes in accordance with the principles of the present invention.

In particular, the *ca* A/Leningrad/47 virus is used as a model for the introduction of mutations. Klimov, A.I. et al., *Virol.* 186:795-797 (1992). The *ca*  
30 A/Leningrad/47 virus has been chosen as a model because 1) differences between the *wt* A/Leningrad, A/Leningrad/17, and A/Leningrad/47 viruses are published knowledge and they are one of the few H2N2 viruses sequenced and listed in GenBank; 2) these differences will not be lethal mutations; 3) these differences probably will not interfere with growth; 4) one or several of them may introduce  
35 another temperature sensitive (*ts*) lesion into the *ca* A/AA/6/60 genome. Since the PA, M, and NS genes of the *ca* and the *wt* 2(3) A/AA/6/60 viruses are identical, those three



genes have been targeted for mutation. The *ca* A/Leningrad/47 PA gene has three differences from the *wt* A/Leningrad virus; the M gene has two differences and a *ts* lesion; and the NS gene has one difference and a *ts* lesion. The *ca* A/AA/6/60 virus has the nucleotides at these positions of the *wt* A/Leningrad virus, with the exception of 969 in the matrix gene. Because a helper virus is available which will facilitate the selection of clones bearing a mutated NS gene, that gene is mutated first and rescued using the techniques of reverse genetics known to those in the art. Nucleotide 798 of the *ca* NS gene will be mutated from guanine to adenine, coding for methionine to isoleucine in NS2. Although this nucleotide has not been definitively identified as responsible for the *ts* lesion residing on the NS gene of *ca* Leningrad, it is the only difference from the *wt* Leningrad sequence. After the mutation has been successfully rescued, the mutated *ca* A/AA/6/60 virus is evaluated for the retention of the *ca* and *ts* markers and for retention of antigenicity, as described above.

#### **SPECIFIC EXAMPLE 9 - VIRAL VECTORS**

The viruses of the present invention are also useful as vectors for foreign proteins. For example, the use of either the HA or NA genes as vectors for foreign viral proteins has been suggested. Li, S. et al. *J. Virol.* 66(1):399-404 (1992) and Castrucci, M.A. et al., *J. Virol.* 67(2):759-764 (1993). H3N2 amino acids and H2N2 amino acids were introduced into the HA of an H1N1 virus, thus constructing a chimeric HA influenza molecule. Li, S. et al., *J. Virol.* 66(1):399-404 (1992). Although foreign viral amino acids or additional amino acids were not introduced into the HA, a chimeric HA can be constructed with antigenic sites important for the current H1N1 and current H3N2 viruses in the same virus. Thus, one virus with a chimeric HA could be given instead of giving a divalent vaccine.

It has been shown that insertion of 28 amino acids into the neuraminidase stalk does not interfere with growth of the virus in eggs; in fact, the longer the stalk, the better it grew. This suggests use of the influenza virus as a vaccine vector to immunize against other unrelated infectious agents. Since the NA is a glycoprotein on the surface of the virus and is one of the two major antigenic proteins for the influenza virus, it may be an excellent site for presentation of a foreign antigenic epitope. Likewise, the *ca* A/AA/6/60 virus may also be used as a vaccine vector, Castrucci, M.A. et al., Abstract 15-4; ASV 12th Annual Meeting, July 10-14 (1993), *i.e.* a vector for the human immunodeficiency virus, HIV.

#### **SPECIFIC EXAMPLE 10 - CLINICAL STUDIES**

As previously stated, many clinical studies have been performed using cold-adapted vaccines. In this study, a live attenuated trivalent combination of vaccines



was evaluated to see if a single intranasal administration of  $\leq 10$  TCID<sub>50</sub> of each vaccine virus could successfully immunize triply seronegative children. A detailed description of this study is also set forth in Belshe, R.B. et al., *J. Infect. Dis.* 165:727-732 (1992).

5       **Materials and Methods.** The cold-recombinant (CR) influenza A vaccines and the CR influenza B vaccine included in the trivalent vaccine were derived from cold-adapted parent strains of influenza using methods previously described. Maassab, H.F., *J. Immunol.* 102:728-732 (1969); Cox, N.J. et al., *Virology* 97:190-194 (1979); Maassab, H.F. et al., *Virology* 130:342-350 (1983); Maassab, H.F. et al., *J. Infect. Dis.* 10 146:780-790 (1982); Donabedian, A.M. et al., *Microb. Pathog.* 3:97-108 (1987). Influenza A/Kawasaki/9/86 (H1N1) and influenza A/Korea/1/82 (H3N2) were derived from the cold-adapted influenza A/Ann Arbor/6/60 parent virus, while influenza B/Texas/1/84 was produced from influenza B/Ann Arbor/1/66 cold-adapted parent virus. The vaccine viruses, designated CR125 (H1N1), CR59 (H3N2), and CRB-87, 15 possessed the six internal genes of their parent cold-adapted virus, A/Ann Arbor/6/60 or B/Ann Arbor/1/66, and the hemagglutinin and neuraminidase genes of their respective wild type strains. Vaccinees received 0.5 ml of the cold-adapted trivalent influenza vaccine consisting of a mixture of CR125 and CRB-87, each diluted 1:100, and CR59 diluted 1:50. To ensure that an equal titer of each viral strain was 20 incorporated into the trivalent vaccine, each of the three vaccines was diluted separately on the day of vaccination. Subsequently, an equal volume of each was pooled to make the vaccine for administration to the volunteers. Assays were done on an aliquot of each component of the trivalent vaccine to assess the titer of each of the influenza strains incorporated into the vaccine. Titering of vaccine on each of 25 six vaccination dates revealed H1 vaccine to contain a mean of  $10^{5.0}$  TCID<sub>50</sub>, H3 vaccine to contain a mean of  $10^{4.9}$  TCID<sub>50</sub>, and B vaccine to contain a mean of  $10^{5.5}$  TCID<sub>50</sub> per half ml of a vaccine stock before being combined into trivalent vaccine. Thus the final concentration was one-third of the above (H1,  $10^{4.5}$ ; H3,  $10^{4.4}$ ; and B,  $10^{5.0}$  TCID<sub>50</sub>/0.5-ml dose of vaccine).

30       **Vaccination and Clinical Observations.** Healthy infants and children aged 6 months to 13 years were recruited to join the study. Volunteers were randomized to receive vaccine or vaccine diluent as placebo in a double-blinded way. One of every three to four children received placebo.

Children were placed in a supine position and 0.5 ml of vaccine was instilled 35 into the nose as previously described. Belshe, R.B. et al. *J. Infect. Dis.* 149:735-740 (1984); Anderson, E.L. et al., *J. Clin. Microbiol.* 27: 909-914 (1989). After vaccination,



the children were observed in their homes for 11 days by the vaccine center nursing staff with daily sampling by nasopharyngeal swabbing for isolation of influenza virus. Serum for antibody determinations was obtained on days 0 and 28-31. One post-vaccine serum sample was obtained on day 60.

- 5           Potential adverse reactions were defined as: (1) fever, rectal temperature > 38.3°C (infants and young children) or oral temperature > 37.8°C (older children); (2) cough, two or more episodes noted during examination visits on 2 consecutive days; (3) rhinorrhea, fluid or mucus exiting nostrils on 2 consecutive days; (4) wheeze, sustained musical sound during expiration and confirmed by a physician investigator;
- 10       (5) otitis media, red, immovable ear drum diagnosed by a physician using pneumotoscopy; (6) rhonchi, continuous low-pitched sound heard by auscultation of lung fields; (7) rales, discontinuous, interrupted explosive sounds, fine or coarse crackles heard by auscultation of lung fields and confirmed by a physician; and (8) pneumonia, a new alveolar consolidation seen radiographically.

- 15           **Laboratory Studies.** Serologic tests for antibody to each vaccine strain were assayed by hemagglutination inhibition (HAI) and ELISA. HAI assays used homologous, tissue-culture-grown antigen for each of the vaccine strains in the trivalent vaccine as previously described. World Health Organization, "The hemagglutination inhibition test for influenza virus." U.S. Department of Health,
- 20       Education and Welfare Procedure Manual, Atlanta:Center for Disease Control (1975). Prevacination immune status of the vaccinees was based on HAI titers; a titer <1:4 was considered seronegative. Purified hemagglutinin from heterologous influenza strains, consisting of influenza Taiwan (A/H1N1), influenza Shanghai (A/H3N2), and influenza B/Yamagata (Connaught Laboratories, Swiftwater, PA), was used for the
- 25       ELISA. Briefly, microtiter plates (Dynatech, Chantilly, VA) were coated with antigen (1 µg/ml) overnight at 4°C. The remaining steps of the ELISA procedure were done the next day as follows: (1) antigen was removed but the plates were not washed; (2) plates were blocked with 0.1% bovine serum albumin in PBS and washed with PBS-Tween; (3) four-fold dilutions of test samples were added to the plates and the plates
- 30       were incubated at 37°C for 2 hr; (4) after plates were washed with PBS-Tween, goat anti-human IgG was added for a 2 hr incubation at 37°C; and (5) plates were washed, developed using a phosphatase substrate kit (Kirkegaard & Perry, Gaithersburg, MD), and read in a microtiter plate reader after 30 min for IgG and 90 min for IgA. An antibody response was defined as a seroconversion by HAI or ELISA (<1:4 to ≥1:8
- 35       by HAI; <1:20 to ≥ 1:20 by ELISA) or as a four-fold increase in titer.



Viral shedding was monitored by isolation in cell-culture tubes of primary rhesus monkey kidney (RhMK) cells as previously described. Belshe, R.B. et al., *J. Infect. Dis.* 150:834-840 (1984). Cell cultures were incubated at 32°C for 14 days. Hemadsorption of monolayers with 0.4% guinea pig erythrocytes was done on days 5, 9 and 14. In addition, some specimens were inoculated into RhMK tubes containing combinations of polyvalent antiserum specific for two of the three subtypes to permit selective growth of the third subtype. Viral subtype was identified by HAI or by indirect immunofluorescence using monoclonal antibodies (see below). Harmon, N.W. et al., *Influenza Viruses* in "Diagnostic Procedures for Viral Rickettsial and Chlamydial Infections." Schmidt, N.J. et al. (eds.) Washington, D.C.: American Public Health Association 651-653 (1989); Riggs, R.L., *Immunofluorescence Staining* in "Diagnostic Procedures for Viral Rickettsial and Chlamydial Infections." Schmidt, N.J. et al. (eds.) Washington, D.C.: American Public Health Association 651-653 (1989).

To enumerate the viral subtypes shed by each vaccinee, plaque assays were done using subtype-specific monoclonal antibodies in an immunoperoxidase-staining procedure. Confluent monolayers of RhMK cells in 24-well plates were rinsed with sterile PBS, pH 7.2, and then infected in triplicate with 0.2 ml/well of specimen. After absorption for 1 h at 33°C, each well was overlaid with L-15 medium (Whittaker M.A. Bioproducts, Walkersville, MD) containing 1% agarose (SeaKem; FMC Bioproducts, Rockland, ME), 200 mM L-glutamine (Whittaker M.A. Products), and 50 µg/ml gentamicin. Infected plates were incubated at 33°C for 3 days. Subsequently, plates were fixed, the agarose overlay was removed, and the plates were stained by a modification of an immunoperoxidase procedure developed by William Gruber (Department of Pediatrics, Vanderbilt University, Nashville, TN). Infected monolayers were first fixed sequentially with 80% and 100% methanol for 15 min at 4°C, and then were overlaid with 5% skim milk (Difco, Detroit) in PBS for 30 min at 37°C. After removal of the skim milk, each well was overlaid with 0.2 ml of subtype-specific monoclonal antibody diluted 1:2000 (v/v, in PBS for 1 hr at 37°C. Monoclonal antibodies designated as (B/AA/1/86 [B/AA]1/2; A/Mem/2/85 [H3 M2-7]; A/Baylor/11515/82 [H1 AB/28] were provided by Robert Webster, St. Jude Children's Research Hospital (Memphis). After two washes with 5% skim milk, 0.2 ml of peroxidase-conjugated rabbit anti-mouse antibody (1:35, Dako, Carpinteria, CA) was added to each well for 30 min at 37°C. Plates were washed twice with 5% skim milk after which each well was overlaid with 0.2 ml of peroxidase-conjugated swine anti-rabbit antibody (1:90; Dako) for 30 min at 37°C. After two 5% skim milk washes, each well was overlaid with 0.2 ml of AEC substrate (Dako) prepared according to



manufacturer's instructions. Plates were incubated at room temperature until positive control wells showed satisfactory color development (~ 5 min.). Plates were washed with distilled water and read under a dissecting microscope for the presence of red-stained plaques. Uninfected wells were stained in parallel to control for background staining.

**Results.** The clinical and serologic response of vaccinees is summarized in Table 11. As in other trials, some background mild respiratory illness was seen in both vaccinees and controls and was more frequent among children < 12 months old. There was no suggestion of influenza-like symptoms or temporal clustering to suggest that illness was related to vaccine.

The majority of triply seronegative vaccinees exhibited an antibody response to each vaccine component by HAI; fewer antibody rises to H3 and B hemagglutinins (heterologous antigens were used, see Materials and Methods) were detected by ELISA than HAI (Table 11). Of 17 triply seronegative vaccinees, 8 (47%) developed an antibody response to all three strains of the vaccine by HAI or ELISA. Mean postvaccination serum HAI titers were significantly higher for the H3 component than for the other two vaccine strains (Table 11). In contrast to seronegative children, ELISA was more sensitive than HAI at detecting antibody increases in seropositive children (Table 11). Of the 15 seropositive children, by ELISA 4 (27%) had antibody increases to H1, 4 (27%) to H3, and 5 (33%) to B hemagglutinin.

**TABLE 11**

**Clinical and Serologic Responses After Intranasal Vaccination  
With Cold-Adapted Trivalent Influenza Vaccine**

Finding	Group		
	Seronegative <sup>b</sup> (n = 17)	Seropositive <sup>b</sup> (n = 15)	Control (n = 17)
AGE RANGE, MONTHS	7-23	10-116	6-60
NO. WITH ILLNESS <sup>a</sup>			
Fever	0	2	2
Upper respiratory illness (RI)	12 <sup>c</sup>	5	8
Lower RI	0	0	1
Otitis media	2	4	1
SEROLOGIC RESPONSES TO VACCINE <sup>d</sup>			
H1N1/Kawasaki			



5	Before vaccination	<2	5.3	1.2
	After vaccination	2.7 <sup>e,f</sup>	5.3	1.2
	No. with HAI response	10	0	0
	No. with ELISA response	10	4	NT
	H3N2/Korea			
10	Before vaccination	<2	5.5	1
	After vaccination	4.1 <sup>e,f</sup>	6.1	1.2
	No. with HAI response	12	2	0
	No. with ELISA response	9	4	NT
	B/Texas			
15	Before vaccination	<2	3.4	1
	After vaccination	2.5 <sup>f</sup>	4.2	1.2
	No. with HAI response	8	4	0
	No. with ELISA response	6	5	NT

HAI=hemagglutination inhibition assay; NT=not tested.

<sup>a</sup> Fever, rectal temperature >38.3°C; upper RI, ≥2 consecutive days with rhinorrhea or pharyngitis; lower RI, wheezing or pneumonia; otitis media was diagnosed by a pediatrician.

<sup>b</sup> Seronegative (HAI <1:4) or seropositive (HAI ≥ 1:4) to all three strains of virus. Two children were vaccinated and were doubly or singly seronegative; they are not included in the analysis.

<sup>c</sup> Significantly more rhinorrhea than seropositive vaccinees (12 of 17 vs. 5 of 17,  $\chi^2 = 5.8$ ;  $P < 0.05$ ) but not significant when compared to controls (Fisher's exact test,  $P = 0.14$ ).

<sup>d</sup> Antibody response defined as four-fold increase; for negative volunteers a titer rise from <1:4 to ≥1:8 by HAI or ≥1:20 by ELISA.

<sup>e</sup>  $P < 0.03$ , Student's *t* test.

<sup>f</sup>  $P < 0.02$ , Student's *t* test.

As shown in Table 12, viral shedding was observed in most seronegative volunteers and occurred significantly more often in seronegative recipients than in seropositive recipients ( $P \leq 0.02$  for all comparisons between seronegatives and seropositives stratified by viral subtype). Sixteen of seventeen seronegative vaccinees shed at least one strain of virus; one vaccinee who failed to shed vaccine was infected with coxsackie B2 virus. Shedding of H1 and H3 was first observed 1 day after



vaccination while type B shedding began on day 2. The number of children shedding vaccine virus peaked on day 4 for H1, on day 6 for H3, and day 5 for B.

**TABLE 12**

**5                      Viral Shedding After Intranasal Vaccination  
                            With Cold-Adapted Trivalent Influenza Vaccine**

	Vaccine	Subjects	
		Seronegative <sup>a</sup>	Seropositive <sup>a</sup>
10	H1N1/Kawasaki		
	No. shedding/no. infected with vaccine virus <sup>b</sup>	10/12	2/5
	Mean duration (days)	7.8	9
	Mean peak titer (pfu/ml)	12	NT
15	H3N2/Korea		
	No. shedding/no. infected with vaccine virus <sup>b</sup>	13/13	2/4
	Mean duration (days)	8.8	6.5
	Mean peak titer (pfu/ml)	74	NT
20	B/Texas		
	No. shedding/no. infected with vaccine virus <sup>b</sup>	11/13	2/6
	Mean duration (days)	9.4	3.5
	Mean peak titer (pfu/ml)	41	NT

25                      Eleven seronegative subjects were infected with all three vaccine viruses;  
NT = not tested.

30                      <sup>a</sup> Hemagglutination inhibition assay seronegative and seropositive values, respectively,  
were <1:4 or ≥1:4.

<sup>b</sup> Indicated by viral shedding or antibody response by hemagglutination inhibition assay or by ELISA.

35                      Plaque assays to quantitate each subtype shed by seronegative vaccinees  
were done on samples from 15 of 17 volunteers (Table 11). The minimum titer  
detectable by plaque assay was 5 pfu/ml. Specimens positive by tube culture but  
negative by plaque assay were considered to have a titer < 5 pfu/ml. The highest  
mean viral titer was observed for H3 (74 pfu/ml), which was significantly higher than  
40                      that of H1 (12 pfu/ml;  $p < 0.02$ , Student's *t* test). The highest titers of H1 were shed



early, on days 3 and 4 after vaccination. Peak H3 and B titers were found on days 7 and 4 after vaccination, respectively.

Overall, 12 (71%), 13 (76%), and 13 (76%) of seronegative children were infected by H1N1, H3N2, or B vaccine viruses, respectively, as indicated by viral shedding or by HAI or ELISA antibody responses (Table 12). Eleven (65%) were infected by all three strains. Among seropositive children five (33%), four (27%), and six (40%) were infected by H1N1, H3N2, or B vaccine viral strains, respectively, as indicated by viral shedding or by HAI or ELISA antibody responses. None of the seropositive children was infected by all three vaccine viruses.

10 Those skilled in the art can now appreciate from the foregoing description that the broad teachings of the present invention can be implemented in a variety of forms. Therefore, while this invention has been described in connection with particular examples thereof, the true scope of the invention should not be so limited since other modifications will become apparent to the skilled practitioner upon a study of the  
15 drawings, specification and following claims.

All applications and publications cited herein are incorporated by reference.





SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Maassab, Hunein F  
Herlocher, Martha L

(ii) TITLE OF INVENTION: Cold-adapted Influenza Virus

(iii) NUMBER OF SEQUENCES: 40

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Anna M Lewak  
(B) STREET: 5445 Corporate Drive  
(C) CITY: Troy  
(D) STATE: MI  
(E) COUNTRY: USA  
(F) ZIP: 48098

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US  
(B) FILING DATE:  
(C) CLASSIFICATION:



(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Lewak, Anna M
- (B) REGISTRATION NUMBER: 33006
- (C) REFERENCE/DOCKET NUMBER: 2115-00257

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 313-641-1600
- (B) TELEFAX: 313-641-0270

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 890 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: cold-adapted "Master Strain" A/Ann Arbor/6/60 7PI  
(H2N2)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: NS

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 27..56
- (D) OTHER INFORMATION: /product= "nonstructural protein NS2"  
/gene= "NS"  
/note= "nonstructural protein NS2"  
/citation= ([1][2])



(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(483, "a")
- (D) OTHER INFORMATION: /note= "a in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 529..861
- (D) OTHER INFORMATION: /product= "nonstructural protein NS2"  
/gene= "NS"  
/note= "nonstructural protein NS2"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(813, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(27..56, 529..861)
- (D) OTHER INFORMATION: /product= "nonstructural protein NS2"  
/gene= "NS"  
/note= "nonstructural protein NS2"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 27..677



(D) OTHER INFORMATION: /product= "nonstructural protein NS1"  
/gene= "NS"  
/note= "nonstructural protein NS1"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G

(B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus

(C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA

(G) DATE: 1993

(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 890

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Cox, N J  
Kitame, F  
Kendal, A P  
Maassab, H F  
Naeve, C

(B) TITLE: Identification of sequence changes in the cold-adapted  
live attenuated influenza vaccine strain, A/Ann  
Arbor/6/60(H2N2)

(C) JOURNAL: Virology

(D) VOLUME: 167

(F) PAGES: 554-567

(G) DATE: 1988

(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 890

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGCAAAAGCA GGGUGACAAA GACAUA AUG GAU CCU AAC ACU GUG UCA AGC UUU  
Met Asp Pro Asn Thr Val Ser Ser Phe  
1 5



[illegible]



GCAAGCCUUA CAGCUGCUAU UUGAAGUGGA ACAAGAGAUU AGAACUUUCU CGUUUCAGCU 857  
UAUUUAAUGA UAAAAACAC CCUUGUUUCU ACU 890

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 217 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asp Pro Asn Thr Val Ser Ser Phe Gln Val Asp Cys Phe Leu Trp  
1 5 10 15  
His Val Arg Lys Gln Val Ala Asp Gln Glu Leu Gly Asp Ala Pro Phe  
20 25 30  
Leu Asp Arg Leu Arg Arg Asp Gln Lys Ser Leu Arg Gly Arg Gly Ser  
35 40 45  
Thr Leu Gly Leu Asn Ile Glu Thr Ala Thr Arg Val Gly Lys Gln Ile  
50 55 60  
Val Glu Arg Ile Leu Lys Glu Glu Ser Asp Glu Ala Leu Lys Met Thr  
65 70 75 80  
Met Ala Ser Ala Pro Ala Ser Arg Tyr Leu Thr Asp Met Thr Ile Glu  
85 90 95  
Glu Met Ser Arg Asp Trp Phe Met Leu Met Pro Lys Gln Lys Val Ala  
100 105 110  
Gly Pro Leu Cys Ile Arg Met Asp Gln Ala Ile Met Asp Lys Asn Ile  
115 120 125  
Ile Leu Lys Ala Asn Phe Ser Val Ile Phe Asp Arg Leu Glu Thr Leu  
130 135 140  
Ile Leu Leu Arg Ala Phe Thr Glu Thr Gly Ala Ile Val Gly Glu Ile  
145 150 155 160  
Ser Pro Leu Pro Ser Leu Pro Gly His Thr Asn Glu Asp Val Lys Asn  
165 170 175



Ala Ile Gly Val Leu Ile Gly Gly Leu Glu Trp Asn Asp Asn Thr Val  
 180 185 190  
 Arg Val Ser Lys Thr Leu Gln Arg Phe Ala Trp Arg Ser Ser Asp Glu  
 195 200 205  
 Asn Gly Arg Pro Pro Leu Thr Pro Lys  
 210 215

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 418 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 27..389
- (D) OTHER INFORMATION: /product= "Nonstructural protein 2"  
 /gene= "NS2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGCAAAAGCA GGGUGACAAA GACAUA AUG GAU CCU AAC ACU GUG UCA AGC UUU	53
Met Asp Pro Asn Thr Val Ser Ser Phe	
1 5	
CAG GAC AUA CUA AUG AGG AUG UCA AAA AUG CAA UUG GGG UCC UCA UCG	101
Gln Asp Ile Leu Met Arg Met Ser Lys Met Gln Leu Gly Ser Ser Ser	
10 15 20 25	
GAG GAC UUG AAU GGA AUG AUA ACA CAG UUC GAG UCU CUA AAA CUC UAC	149
Glu Asp Leu Asn Gly Met Ile Thr Gln Phe Glu Ser Leu Lys Leu Tyr	
30 35 40	
AGA GAU UCG CUU GGA GAA GCA GUG AUG AGA AUG GGA GAC CUC CAC UCA	197
Arg Asp Ser Leu Gly Glu Ala Val Met Arg Met Gly Asp Leu His Ser	
45 50 55	



CUC CAA AAU AGA AAC GGA AAA UGG CGA GAA CAA UUA GGU CAA AAG UUC	245
Leu Gln Asn Arg Asn Gly Lys Trp Arg Glu Gln Leu Gly Gln Lys Phe	
60 65 70	
GAA GAA AUA AGA UGG CUG AUU GAA GAA GUG AGA CAC AAA UUG AAG AUA	293
Glu Glu Ile Arg Trp Leu Ile Glu Glu Val Arg His Lys Leu Lys Ile	
75 80 85	
ACA GAG AAU AGU UUU GAG CAA AUA ACA UUU AUG CAA GCC UUA CAG CUG	341
Thr Glu Asn Ser Phe Glu Gln Ile Thr Phe Met Gln Ala Leu Gln Leu	
90 95 100 105	
CUA UUU GAA GUG GAA CAA GAG AUA AGA ACU UUC UCG UUU CAG CUU AUU	389
Leu Phe Glu Val Glu Gln Glu Ile Arg Thr Phe Ser Phe Gln Leu Ile	
110 115 120	
UAAUGAUAAA AAACACCCUU GUUUCUACU	418

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 121 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Pro Asn Thr Val Ser Ser Phe Gln Asp Ile Leu Met Arg Met	
1 5 10 15	
Ser Lys Met Gln Leu Gly Ser Ser Ser Glu Asp Leu Asn Gly Met Ile	
20 25 30	
Thr Gln Phe Glu Ser Leu Lys Leu Tyr Arg Asp Ser Leu Gly Glu Ala	
35 40 45	
Val Met Arg Met Gly Asp Leu His Ser Leu Gln Asn Arg Asn Gly Lys	
50 55 60	
Trp Arg Glu Gln Leu Gly Gln Lys Phe Glu Glu Ile Arg Trp Leu Ile	
65 70 75 80	
Glu Glu Val Arg His Lys Leu Lys Ile Thr Glu Asn Ser Phe Glu Gln	
85 90 95	



Ile Thr Phe Met Gln Ala Leu Gln Leu Leu Phe Glu Val Glu Gln Glu  
          100                          105                          110

Ile Arg Thr Phe Ser Phe Gln Leu Ile  
          115                          120

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1027 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: cold-adapted "Master Strain" A/Ann Arbor/6/60 7PI  
(H2N2)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: M

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 26..51
- (D) OTHER INFORMATION: /product= "matrix protein M2"  
                          /gene= "M"  
                          /note= "matrix protein M2"  
                          /citation= ([1][2])



(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION: 740..1004

(D) OTHER INFORMATION: /product= "matrix protein M2" /gene= "M"  
/note= "matrix protein M2"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(969, "u")

(D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: join(26..51, 740..1004)

(D) OTHER INFORMATION: /product= "matrix protein M2"  
/gene= "M"  
/note= "matrix protein M2"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 26..781

(D) OTHER INFORMATION: /product= "matrix protein M1"  
/gene= "M"  
/note= "matrix protein M1"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G

(B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus



(C) JOURNAL: Proceedings of the National Academy of Sciences of the USA

(G) DATE: 1993

(K) RELEVANT RESIDUES IN SEQ ID NO:5: FROM 1 TO 1027

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Cox, N J  
Kitame, F  
Kendal, A P  
Maassab, H F  
Naeve, C

(B) TITLE: Identification of sequence changes in the cold-adapted live attenuated influenza vaccine strain, A/Ann Arbor/6/60(H2N2)

(C) JOURNAL: Virology

(D) VOLUME: 167

(F) PAGES: 554-557

(G) DATE: 1988

(K) RELEVANT RESIDUES IN SEQ ID NO:5: FROM 1 TO 1027

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGCAAAAGCA	GGUAGAUUU	GAAAG	AUG	AGU	CUU	CUA	ACC	GAG	GUC	GAA	ACG	52				
			Met	Ser	Leu	Leu	Thr	Glu	Val	Glu	Thr					
			1				5									
UAC	GUU	CUC	UCU	AUC	AUC	CCG	UCA	GGC	CCC	CUC	AAA	GCC	GAG	AUC	GCA	100
Tyr	Val	Leu	Ser	Ile	Ile	Pro	Ser	Gly	Pro	Leu	Lys	Ala	Glu	Ile	Ala	
10				15				20				25				
CAG	AGA	CUU	GAA	GAU	GUC	UUU	GCU	GGG	AAA	AAC	ACC	GAU	CUU	GAG	GCU	148
Gln	Arg	Leu	Glu	Asp	Val	Phe	Ala	Gly	Lys	Asn	Thr	Asp	Leu	Glu	Ala	
				30				35					40			
CUC	AUG	GAA	UGG	CUA	AAG	ACA	AGA	CCA	AUC	CUG	UCA	CCU	CUG	ACU	AAG	196
Leu	Met	Glu	Trp	Leu	Lys	Thr	Arg	Pro	Ile	Leu	Ser	Pro	Leu	Thr	Lys	
			45					50					55			
GGG	AUU	UUG	GGA	UUU	GUA	UUC	ACG	CUC	ACC	GUG	CCC	AGU	GAG	CGA	GGA	244
Gly	Ile	Leu	Gly	Phe	Val	Phe	Thr	Leu	Thr	Val	Pro	Ser	Glu	Arg	Gly	
			60				65					70				



[illegible]



(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 252 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Ser	Leu	Leu	Thr	Glu	Val	Glu	Thr	Tyr	Val	Leu	Ser	Ile	Ile	Pro
1				5					10					15	
Ser	Gly	Pro	Leu	Lys	Ala	Glu	Ile	Ala	Gln	Arg	Leu	Glu	Asp	Val	Phe
			20					25					30		
Ala	Gly	Lys	Asn	Thr	Asp	Leu	Glu	Ala	Leu	Met	Glu	Trp	Leu	Lys	Thr
		35					40					45			
Arg	Pro	Ile	Leu	Ser	Pro	Leu	Thr	Lys	Gly	Ile	Leu	Gly	Phe	Val	Phe
	50					55					60				
Thr	Leu	Thr	Val	Pro	Ser	Glu	Arg	Gly	Leu	Gln	Arg	Arg	Arg	Phe	Val
65					70					75					80
Gln	Asn	Ala	Leu	Asn	Gly	Asn	Gly	Asp	Pro	Asn	Asn	Met	Asp	Arg	Ala
				85					90					95	
Val	Lys	Leu	Tyr	Arg	Lys	Leu	Lys	Arg	Glu	Ile	Thr	Phe	His	Gly	Ala
			100					105					110		
Lys	Glu	Ile	Ala	Leu	Ser	Tyr	Ser	Ala	Gly	Ala	Leu	Ala	Ser	Cys	Met
		115				120						125			
Gly	Leu	Ile	Tyr	Asn	Arg	Met	Gly	Ala	Val	Thr	Thr	Glu	Val	Val	Leu
	130					135					140				
Gly	Leu	Val	Cys	Ala	Thr	Cys	Glu	Gln	Ile	Ala	Asp	Ser	Gln	His	Arg
145					150					155					160
Ser	His	Arg	Gln	Met	Val	Thr	Thr	Thr	Asn	Pro	Leu	Ile	Arg	His	Glu
				165					170					175	
Asn	Arg	Met	Val	Leu	Ala	Ser	Thr	Thr	Ala	Lys	Ala	Met	Glu	Gln	Met
			180					185					190		
Ala	Gly	Ser	Ser	Glu	Gln	Ala	Ala	Glu	Ala	Met	Glu	Val	Ala	Ser	Gln
		195				200						205			



Ala Arg Gln Met Val Gln Ala Met Arg Val Ile Gly Thr His Pro Ser  
 210 215 220  
 Ser Ser Ala Gly Leu Lys Asn Asp Leu Leu Glu Asn Leu Gln Ala Tyr  
 225 230 235 240  
 Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys  
 245 250

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 339 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 26..316
- (D) OTHER INFORMATION: /product= "Matrix M2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AGCAAAAGCA GGUAGAUUU GAAAG AUG AGU CUU CUA ACC GAG GUC GAA ACG	52
Met Ser Leu Leu Thr Glu Val Glu Thr	
1 5	
CCU AUC AGA AAC GAA UGG GGG UGC AGA UGC AAC GAU UCA AGU GAC CCU	100
Pro Ile Arg Asn Glu Trp Gly Cys Arg Cys Asn Asp Ser Ser Asp Pro	
10 15 20 25	
CUU GUU GUU GCC GCG AGU AUC AUU GGG AUC UUG CAC UUG AUA UUG UGG	148
Leu Val Val Ala Ala Ser Ile Ile Gly Ile Leu His Leu Ile Leu Trp	
30 35 40	
AUU CUU GAU CAU CUU UUU UUC AAA UGC AUU UAU CGC UUC UUU AAA CAC	196
Ile Leu Asp His Leu Phe Phe Lys Cys Ile Tyr Arg Phe Phe Lys His	
45 50 55	



GGU CUG AAA AGA GGG CCU UCU ACG GAA GGA GUA CCA GAG UCU AUG AGG	244
Gly Leu Lys Arg Gly Pro Ser Thr Glu Gly Val Pro Glu Ser Met Arg	
60 65 70	
GAA GAA UAU CGA AAG GAA CAG CAG AGU GCU GUG GAU UCU GAC GAU AGU	292
Glu Glu Tyr Arg Lys Glu Gln Gln Ser Ala Val Asp Ser Asp Asp Ser	
75 80 85	
CAU UUU GUC AGC AUA GAG CUG GAG UAAAAACUA CCUUGUUUCU ACU	339
His Phe Val Ser Ile Glu Leu Glu	
90 95	

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ser Leu Leu Thr Glu Val Glu Thr Pro Ile Arg Asn Glu Trp Gly	
1 5 10 15	
Cys Arg Cys Asn Asp Ser Ser Asp Pro Leu Val Val Ala Ala Ser Ile	
20 25 30	
Ile Gly Ile Leu His Leu Ile Leu Trp Ile Leu Asp His Leu Phe Phe	
35 40 45	
Lys Cys Ile Tyr Arg Phe Phe Lys His Gly Leu Lys Arg Gly Pro Ser	
50 55 60	
Thr Glu Gly Val Pro Glu Ser Met Arg Glu Glu Tyr Arg Lys Glu Gln	
65 70 75 80	
Gln Ser Ala Val Asp Ser Asp Asp Ser His Phe Val Ser Ile Glu Leu	
85 90 95	
Glu	



(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1566 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: cold-adapted "Master Strain" A/Ann Arbor/6/60 7PI (H2N2)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: NP

(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(113, "c")
- (D) OTHER INFORMATION: /note= "c in ca "master" strain; a in wt2(3); a in 1988 reported ca vaccine strain (manuscript), but c reported in 1988 genbank"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(146, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in wt2(3)"  
/citation= ([1][2])



(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(627, "c")
- (D) OTHER INFORMATION: /note= "c in ca "master" strain and in  
wt2(3); a in 1988 reported ca vaccine  
strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(909, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); c in 1988 reported ca vaccine  
strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(1550, "a")
- (D) OTHER INFORMATION: /note= "a in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 46..1539
- (D) OTHER INFORMATION: /product= "Nucleoprotein"  
/gene= "NP"  
/note= "nucleoprotein"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R W
- (B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus



(C) JOURNAL: Proceedings of the National Academy of Sciences of the USA

(G) DATE: 1993

(K) RELEVANT RESIDUES IN SEQ ID NO:9: FROM 1 TO 1566

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Cox, N J  
Kitame, F  
Kendal, A P  
Maassab, H F  
Naeve, C

(B) TITLE: Identification of sequence changes in the cold-adapted live attenuated influenza vaccine strain, A/Ann Arbor/6/60 (H2N2)

(C) JOURNAL: Virology

(D) VOLUME: 167

(F) PAGES: 554-567

(G) DATE: 1988

(K) RELEVANT RESIDUES IN SEQ ID NO:9: FROM 1 TO 1566

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGCAAAAGCA GGGUAGAUAA UCACUCACUG AGUGACAUCA AAAUC AUG GCG UCC	54
Met Ala Ser	
1	
CAA GGC ACC AAA CGG UCU UAU GAA CAG AUG GAA ACU GAU GGG GAA CGC	102
Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp Gly Glu Arg	
5 10 15	
CAG AAU GCA ACU GAA AUC AGA GCA UCC GUC GGG AAG AUG AUU GGU GGA	150
Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met Ile Gly Gly	
20 25 30 35	
AUU GGA CGA UUC UAC AUC CAA AUG UGC ACC GAA CUU AAA CUC AGU GAU	198
Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys Leu Ser Asp	
40 45 50	
UAU GAG GGG CGG CUG AUC CAG AAC AGC UUA ACA AUA GAG AGA AUG GUG	246
Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu Arg Met Val	
55 60 65	



CUC Leu	UCU Ser	GCU Ala 70	UUU Phe	GAC Asp	GAG Glu	AGG Arg	AGG Arg 75	AAU Asn	AAA Lys	UAU Tyr	CUG Leu	GAA Glu 80	GAA Glu	CAU His	CCC Pro	294
AGC Ser	GCG Ala 85	GGG Gly	AAG Lys	GAU Asp	CCU Pro	AAG Lys 90	AAA Lys	ACU Thr	GGA Gly	GGA Gly	CCC Pro 95	AUA Ile	UAC Tyr	AAG Lys	AGA Arg	342
GUA Val 100	GAU Asp	GGA Gly	AAG Lys	UGG Trp	AUG Met 105	AGG Arg	GAA Glu	CUC Leu	GUC Val	CUU Leu 110	UAU Tyr	GAC Asp	AAA Lys	GAA Glu	GAA Glu 115	390
AUA Ile	AGG Arg	CGA Arg	AUC Ile	UGG Trp 120	CGC Arg	CAA Gln	GCU Ala	AAU Asn	AAU Asn 125	GGU Gly	GAU Asp	GAU Asp	GCA Ala	ACA Thr 130	GCU Ala	438
GGU Gly	CUG Leu	ACU Thr	CAC His 135	AUG Met	AUG Met	AUC Ile	UGG Trp	CAU His 140	UCC Ser	AAU Asn	UUG Leu	AAU Asn	GAU Asp 145	ACA Thr	ACA Thr	486
UAC Tyr	CAG Gln 150	AGG Arg	ACA Thr	AGA Arg	GCU Ala	CUU Leu	GUU Val 155	CGC Arg	ACC Thr	GGA Gly	AUG Met 160	GAU Asp	CCC Pro	AGG Arg	AUG Met	534
UGC Cys 165	UCU Ser	UUG Leu	AUG Met	CAG Gln	GGU Gly	UCG Ser 170	ACU Thr	CUC Leu	CCU Pro	AGG Arg	AGG Arg 175	UCU Ser	GGA Gly	GCC Ala	GCA Ala	582
GGC Gly 180	GCU Ala	GCA Ala	GUC Val	AAA Lys	GGA Gly 185	GUU Val	GGG Gly	ACA Thr	AUG Met	GUG Val 190	AUG Met	GAG Glu	UUG Leu	AUC Ile	AGG Arg 195	630
AUG Met	AUC Ile	AAA Lys	CGU Arg	GGG Gly 200	AUC Ile	AAU Asn	GAU Asp	CGG Arg	AAC Asn 205	UUC Phe	UGG Trp	AGA Arg	GGU Gly	GAG Glu 210	AAU Asn	678
GGG Gly	CGG Arg	AAA Lys	ACA Thr 215	AGG Arg	AAU Asn	GCU Ala	UAU Tyr	GAG Glu 220	AGA Arg	AUG Met	UGC Cys	AAC Asn	AUU Ile	CUC Leu	AAA Lys	726
GGA Gly	AAA Lys	UUU Phe 230	CAA Gln	ACA Thr	GCU Ala	GCA Ala	CAA Gln 235	AGA Arg	GCA Ala	AUG Met	AUG Met	GAU Asp 240	CAA Gln	GUG Val	AGA Arg	774
GAA Glu 245	AGC Ser	CGG Arg	AAC Asn	CCA Pro	GGA Gly	AAU Asn 250	GCU Ala	GAG Glu	AUC Ile	GAA Glu	GAU Asp 255	CUC Leu	AUC Ile	UUU Phe	CUG Leu	822
GCA Ala 260	CGG Arg	UCU Ser	GCA Ala	CUC Leu	AUA Ile 265	UUG Leu	AGA Arg	GGG Gly	UCA Ser	GUU Val 270	GCU Ala	CAC His	AAA Lys	UCU Ser	UGU Cys 275	870
CUG Leu	CCU Pro	GCC Ala	UGU Cys	GUG Val 280	UAU Tyr	GGA Gly	CCU Pro	GCC Ala	GUA Val 285	GCC Ala	AGU Ser	GGG Gly	UAC Tyr	GAC Asp 290	UUC Phe	918



GAA AAA GAG GGA UAC UCU UUA GUA GGG AUA GAC CCU UUC AAA CUG CUU Glu Lys Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe Lys Leu Leu 295 300 305	966
CAA AAC AGC CAA GUA UAC AGC CUA AUC AGA CCG AAU GAG AAU CCA GCA Gln Asn Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu Asn Pro Ala 310 315 320	1014
CAC AAG AGU CAG CUG GUG UGG AUG GCA UGC AAU UCU GCU GCA UUU GAA His Lys Ser Gln Leu Val Trp Met Ala Cys Asn Ser Ala Ala Phe Glu 325 330 335	1062
GAU CUA AGA GUA UCA AGC UUC AUC AGA GGG ACC AAA GUA AUC CCA AGG Asp Leu Arg Val Ser Ser Phe Ile Arg Gly Thr Lys Val Ile Pro Arg 340 345 350 355	1110
GGG AAA CUU UCC ACU AGA GGA GUA CAA AUU GCU UCA AAU GAA AAC AUG Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn Glu Asn Met 360 365 370	1158
GAU ACU AUG GGA UCA AGU ACU CUU GAA CUG AGA AGC AGG UAC UGG GCC Asp Thr Met Gly Ser Ser Thr Leu Glu Leu Arg Ser Arg Tyr Trp Ala 375 380 385	1206
AUA AGG ACC AGA AGU GGA GGA AAC ACU AAU CAA CAG AGG GCC UCU GCA Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg Ala Ser Ala 390 395 400	1254
GGU CAA AUC AGU GUA CAA CCU ACG UUU UCU GUG CAA AGA AAC CUC CCA Gly Gln Ile Ser Val Gln Pro Thr Phe Ser Val Gln Arg Asn Leu Pro 405 410 415	1302
UUU GAC AAA CCA ACC AUC AUG GCA GCA UUC ACU GGG AAU GCA GAG GGA Phe Asp Lys Pro Thr Ile Met Ala Ala Phe Thr Gly Asn Ala Glu Gly 420 425 430 435	1350
AGA ACA UCA GAC AUG AGG GCA GAA AUC AUA AGG AUG AUG GAA GGU GCA Arg Thr Ser Asp Met Arg Ala Glu Ile Ile Arg Met Met Glu Gly Ala 440 445 450	1398
AAA CCA GAA GAA GUG UCC UUC CAG GGG CGG GGA GUC UUC GAG CUC UCG Lys Pro Glu Glu Val Ser Phe Gln Gly Arg Gly Val Phe Glu Leu Ser 455 460 465	1446
GAC GAA AAG GCA ACG AAC CCG AUC GUG CCC UCU UUU GAC AUG AGU AAU Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp Met Ser Asn 470 475 480	1494
GAA GGA UCU UAU UUC UUC GGA GAC AAU GCA GAG GAG UAC GAC AAU Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr Asp Asn 485 490 495	1539
UAAGGAAAAA AUACCCUUGU UUCUACU	1566



(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 498 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp  
1 5 10 15  
Gly Glu Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met  
20 25 30  
Ile Gly Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys  
35 40 45  
Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu  
50 55 60  
Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu  
65 70 75 80  
Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile  
85 90 95  
Tyr Lys Arg Val Asp Gly Lys Trp Met Arg Glu Leu Val Leu Tyr Asp  
100 105 110  
Lys Glu Glu Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Asp Asp  
115 120 125  
Ala Thr Ala Gly Leu Thr His Met Met Ile Trp His Ser Asn Leu Asn  
130 135 140  
Asp Thr Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp  
145 150 155 160  
Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser  
165 170 175  
Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Met Glu  
180 185 190  
Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg  
195 200 205



[illegible]



(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2233 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: cold-adapted "Master Strain" A/Ann Arbor/6/60 7PI  
(H2N2)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: PA

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(20, "c")
- (D) OTHER INFORMATION: /note= "c in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(75, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); u in 1988 reported ca vaccine  
strain"  
/citation= ([1][2])



(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(1861, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(2167..2168, "cc")
- (D) OTHER INFORMATION: /note= "cc in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 25..2172
- (D) OTHER INFORMATION: /product= "polymerase acidic protein"  
/gene= "PA"  
/note= "polymerase acidic protein"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G
- (B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus
- (C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA
- (G) DATE: 1993
- (K) RELEVANT RESIDUES IN SEQ ID NO:11: FROM 1 TO 2233



(x) PUBLICATION INFORMATION:

(A) AUTHORS: Cox, N J  
Kitame, F  
Kendal, A P  
Maassab, H F  
Naeve, C

(B) TITLE: Identification of sequence changes in the cold-adapted  
live attenuated influenza strain, A/Ann  
Arbor/6/60(H2N2)

(C) JOURNAL: Virology

(D) VOLUME: 167

(F) PAGES: 554-567

(G) DATE: 1988

(K) RELEVANT RESIDUES IN SEQ ID NO:11: FROM 1 TO 2233

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCGAAAGCA GGUACUGAUC CGAA AUG GAA GAU UUU GUG CGA CAA UGC UUC	51
Met Glu Asp Phe Val Arg Gln Cys Phe	
1 5	
AAU CCG AUG AUU GUC GAG CUU GCG GAA AAA GCA AUG AAA GAG UAU GGA	99
Asn Pro Met Ile Val Glu Leu Ala Glu Lys Ala Met Lys Glu Tyr Gly	
10 15 20 25	
GAG GAU CUG AAA AUC GAA ACA AAC AAA UUU GCA GCA AUA UGC ACU CAC	147
Glu Asp Leu Lys Ile Glu Thr Asn Lys Phe Ala Ala Ile Cys Thr His	
30 35 40	
UUG GAA GUA UGC UUC AUG UAU UCA GAU UUU CAU UUC AUC AAU GAG CAA	195
Leu Glu Val Cys Phe Met Tyr Ser Asp Phe His Phe Ile Asn Glu Gln	
45 50 55	
GGC GAG UCA AUA AUA GUA GAG CUU GAU GAU CCA AAU GCA CUU UUG AAG	243
Gly Glu Ser Ile Ile Val Glu Leu Asp Asp Pro Asn Ala Leu Leu Lys	
60 65 70	
CAC AGA UUU GAA AUA AUA GAG GGA AGA GAU CGC ACA AUG GCC UGG ACA	291
His Arg Phe Glu Ile Ile Glu Gly Arg Asp Arg Thr Met Ala Trp Thr	
75 80 85	
GUA GUA AAC AGU AUU UGC AAC ACU ACA GGA GCU GAG AAA CCG AAG UUU	339
Val Val Asn Ser Ile Cys Asn Thr Thr Gly Ala Glu Lys Pro Lys Phe	
90 95 100 105	



CUG	CCA	GAU	UUG	UAU	GAU	UAC	AAG	GAG	AAU	AGA	UUC	AUC	GAG	AUU	GGA	387
Leu	Pro	Asp	Leu	Tyr	Asp	Tyr	Lys	Glu	Asn	Arg	Phe	Ile	Glu	Ile	Gly	
			110					115						120		
GUG	ACA	AGG	AGG	GAA	GUC	CAC	AUA	UAC	UAU	CUU	GAA	AAG	GCC	AAU	AAA	435
Val	Thr	Arg	Arg	Glu	Val	His	Ile	Tyr	Tyr	Leu	Glu	Lys	Ala	Asn	Lys	
			125					130					135			
AUU	AAA	UCU	GAG	AAG	ACA	CAC	AUC	CAC	AUU	UUC	UCA	UUC	ACU	GGG	GAA	483
Ile	Lys	Ser	Glu	Lys	Thr	His	Ile	His	Ile	Phe	Ser	Phe	Thr	Gly	Glu	
		140					145					150				
GAA	AUG	GCC	ACA	AAG	GCC	GAC	UAC	ACU	CUC	GAU	GAG	GAA	AGC	AGG	GCU	531
Glu	Met	Ala	Thr	Lys	Ala	Asp	Tyr	Thr	Leu	Asp	Glu	Glu	Ser	Arg	Ala	
	155					160					165					
AGG	AUC	AAA	ACC	AGA	CUA	UUC	ACC	AUA	AGA	CAA	GAA	AUG	GCU	AGC	AGA	579
Arg	Ile	Lys	Thr	Arg	Leu	Phe	Thr	Ile	Arg	Gln	Glu	Met	Ala	Ser	Arg	
170					175					180					185	
GGC	CUC	UGG	GAU	UCC	UUU	CAU	CAG	UCC	GAA	AGA	GGC	GAA	GAA	ACA	AUU	627
Gly	Leu	Trp	Asp	Ser	Phe	His	Gln	Ser	Glu	Arg	Gly	Glu	Glu	Thr	Ile	
				190					195					200		
GAA	GAA	AGA	UUU	GAA	AUC	ACA	GGG	ACA	AUG	CGC	AGG	CUC	GCC	GAC	CAA	675
Glu	Glu	Arg	Phe	Glu	Ile	Thr	Gly	Thr	Met	Arg	Arg	Leu	Ala	Asp	Gln	
			205					210					215			
AGU	CUC	CCG	CCG	AAC	UUC	UCC	UGC	CUU	GAG	AAU	UUU	AGA	GCC	UAU	GUG	723
Ser	Leu	Pro	Pro	Asn	Phe	Ser	Cys	Leu	Glu	Asn	Phe	Arg	Ala	Tyr	Val	
		220					225					230				
GAU	GGA	UUC	GAA	CCG	AAC	GGC	UAC	AUU	GAG	GGC	AAG	CUU	UCU	CAA	AUG	771
Asp	Gly	Phe	Glu	Pro	Asn	Gly	Tyr	Ile	Glu	Gly	Lys	Leu	Ser	Gln	Met	
	235					240					245					
UCC	AAA	GAA	GUA	AAU	GCU	AAA	AUU	GAA	CCU	UUU	CUG	AAA	ACA	ACA	CCA	819
Ser	Lys	Glu	Val	Asn	Ala	Lys	Ile	Glu	Pro	Phe	Leu	Lys	Thr	Thr	Pro	
250					255					260					265	
AGA	CCA	AUU	AGA	CUU	CCG	GAU	GGG	CCU	CCU	UGU	UCU	CAG	CGG	UCC	AAA	867
Arg	Pro	Ile	Arg	Leu	Pro	Asp	Gly	Pro	Pro	Cys	Ser	Gln	Arg	Ser	Lys	
				270					275					280		
UUC	CUG	CUG	AUG	GAU	GCU	UUA	AAA	UUA	AGC	AUU	GAG	GAC	CCA	AGU	CAC	915
Phe	Leu	Leu	Met	Asp	Ala	Leu	Lys	Leu	Ser	Ile	Glu	Asp	Pro	Ser	His	
			285					290					295			
GAA	GGA	GAG	GGA	AUA	CCA	CUA	UAU	GAU	GCG	AUC	AAG	UGU	AUG	AGA	ACA	963
Glu	Gly	Glu	Gly	Ile	Pro	Leu	Tyr	Asp	Ala	Ile	Lys	Cys	Met	Arg	Thr	
		300					305					310				
UUC	UUU	GGA	UGG	AAA	GAA	CCC	UAU	GUU	GUU	AAA	CCA	CAC	GAA	AAG	GGA	1011
Phe	Phe	Gly	Trp	Lys	Glu	Pro	Tyr	Val	Val	Lys	Pro	His	Glu	Lys	Gly	
	315					320					325					



AUA Ile 330	AAU Asn	CCA Pro	AAU Asn	UAU Tyr	CUG Leu 335	CUG Leu	UCA Ser	UGG Trp	AAG Lys	CAA Gln 340	GUA Val	CUG Leu	GCA Ala	GAA Glu	CUG Leu 345	1059
CAG Gln	GAC Asp	AUU Ile	GAG Glu	AAU Asn 350	GAG Glu	GAG Glu	AAG Lys	AUU Ile	CCA Pro 355	AGA Arg	ACC Thr	AAA Lys	AAC Asn	AUG Met 360	AAG Lys	1107
AAA Lys	ACG Thr	AGU Ser	CAG Gln 365	CUA Leu	AAG Lys	UGG Trp	GCA Ala	CUU Leu 370	GGU Gly	GAG Glu	AAC Asn	AUG Met	GCA Ala 375	CCA Pro	GAG Glu	1155
AAG Lys	GUA Val	GAC Asp 380	UUU Phe	GAC Asp	GAC Asp	UGU Cys	AGA Arg 385	GAU Asp	GUA Val	AGC Ser	GAU Asp	UUG Leu 390	AAG Lys	CAA Gln	UAU Tyr	1203
GAU Asp 395	AGU Ser	GAU Asp	GAA Glu	CCU Pro	GAA Glu	UUA Leu 400	AGG Arg	UCA Ser	CUU Leu	UCA Ser	AGC Ser 405	UGG Trp	AUC Ile	CAG Gln	AAU Asn	1251
GAG Glu 410	UUC Phe	AAC Asn	AAG Lys	GCA Ala	UGC Cys 415	GAG Glu	CUG Leu	ACC Thr	GAU Asp	UCA Ser 420	AUC Ile	UGG Trp	AUA Ile	GAG Glu	CUC Leu 425	1299
GAU Asp	GAG Glu	AUU Ile	GGA Gly	GAA Glu 430	GAU Asp	GUG Val	GCU Ala	CCA Pro	AUU Ile 435	GAA Glu	CAC His	AUU Ile	GCA Ala	AGC Ser 440	AUG Met	1347
AGA Arg	AGG Arg	AAU Asn	UAC Tyr 445	UUC Phe	ACA Thr	GCA Ala	GAG Glu	GUG Val 450	UCU Ser	CAU His	UGC Cys	AGA Arg	GCC Ala 455	ACA Thr	GAA Glu	1395
UAU Tyr	AUA Ile	AUG Met 460	AAG Lys	GGG Gly	GUA Val	UAC Tyr	AUU Ile 465	AAU Asn	ACU Thr	GCC Ala	UUG Leu	CUU Leu 470	AAU Asn	GCA Ala	UCC Ser	1443
UGU Cys 475	GCA Ala	GCA Ala	AUG Met	GAC Asp	GAU Asp	UUC Phe 480	CAA Gln	CUA Leu	AUU Ile	CCC Pro	AUG Met 485	AUA Ile	AGC Ser	AAA Lys	UGU Cys	1491
AGA Arg 490	ACU Thr	AAA Lys	GAG Glu	GGA Gly	AGG Arg 495	CGA Arg	AAG Lys	ACC Thr	AAU Asn	UUA Leu 500	UAU Tyr	GGU Gly	UUC Phe	AUC Ile	AUA Ile 505	1539
AAA Lys	GGA Gly	AGA Arg	UCU Ser	CAC His 510	UUA Leu	AGG Arg	AAU Asn	GAC Asp	ACC Thr 515	GAC Asp	GUG Val	GUA Val	AAC Asn	UUU Phe 520	GUG Val	1587
AGC Ser	AUG Met	GAG Glu	UUU Phe 525	UCU Ser	CUC Leu	ACU Thr	GAC Asp	CCA Pro 530	AGA Arg	CUU Leu	GAG Glu	CCA Pro	CAC His 535	AAA Lys	UGG Trp	1635
GAG Glu	AAG Lys	UAC Tyr 540	UGU Cys	GUU Val	CUU Leu	GAG Glu	AUA Ile 545	GGA Gly	GAU Asp	AUG Met	CUA Leu	CUA Leu 550	AGA Arg	AGU Ser	GCC Ala	1683



AUA Ile 555	GGC Gly	CAG Gln	GUG Val	UCA Ser	AGG Arg	CCC Pro 560	AUG Met	UUC Phe	UUG Leu	UAU Tyr	GUG Val 565	AGG Arg	ACA Thr	AAU Asn	GGA Gly	1731
ACA Thr 570	UCA Ser	AAG Lys	AUU Ile	AAA Lys	AUG Met 575	AAA Lys	UGG Trp	GGA Gly	AUG Met	GAG Glu 580	AUG Met	AGG Arg	CGU Arg	UGC Cys	CUC Leu 585	1779
CUU Leu	CAG Gln	UCA Ser	CUC Leu	CAA Gln 590	CAA Gln	AUC Ile	GAG Glu	AGU Ser	AUG Met 595	AUU Ile	GAA Glu	GCC Ala	GAG Glu	UCC Ser 600	UCU Ser	1827
GUC Val	AAG Lys	GAG Glu	AAA Lys 605	GAC Asp	AUG Met	ACC Thr	AAA Lys	GAG Glu 610	UUU Phe	UUC Phe	GAG Glu	AAU Asn	AAA Lys 615	UCA Ser	GAA Glu	1875
ACA Thr	UGG Trp	CCC Pro 620	AUU Ile	GGA Gly	GAG Glu	UCC Ser	CCC Pro 625	AAA Lys	GGA Gly	GUG Val	GAA Glu	GAA Glu 630	GGU Gly	UCC Ser	AUU Ile	1923
GGG Gly 635	AAG Lys	GUC Val	UGC Cys	AGG Arg	ACU Thr	UUA Leu 640	UUA Leu	GCC Ala	AAG Lys	UCG Ser	GUA Val 645	UUC Phe	AAU Asn	AGC Ser	CUG Leu	1971
UAU Tyr 650	GCA Ala	UCU Ser	CCA Pro	CAA Gln 655	UUA Leu	GAA Glu	GGA Gly	UUU Phe	UCA Ser	GCU Ala 660	GAA Glu	UCA Ser	AGA Arg	AAA Lys	CUG Leu 665	2019
CUU Leu	CUU Leu	GUC Val	GUU Val	CAG Gln 670	GCU Ala	CUU Leu	AGG Arg	GAC Asp	AAU Asn 675	CUU Leu	GAA Glu	CCU Pro	GGG Gly	ACC Thr 680	UUU Phe	2067
GAU Asp	CUU Leu	GGG Gly	GGG Gly 685	CUA Leu	UAU Tyr	GAA Glu	GCA Ala	AUU Ile 690	GAG Glu	GAG Glu	UGC Cys	CUG Leu	AUU Ile 695	AAU Asn	GAU Asp	2115
CCC Pro	UGG Trp	GUU Val 700	UUG Leu	CUU Leu	AAU Asn	GCG Ala	UCU Ser 705	UGG Trp	UUC Phe	AAC Asn	UCC Ser	UUC Phe 710	CUA Leu	ACA Thr	CAU His	2163
GCA Ala 715	CCA Pro	AGA Arg	UAGUUGUGGC AAUGCUACUA UUUGCUAUCC AUACUGUCCA													2212
AAAAAGUACC UUGUUUCUAC U																2233

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 716 amino acids

(B) TYPE: amino acid



(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met	Glu	Asp	Phe	Val	Arg	Gln	Cys	Phe	Asn	Pro	Met	Ile	Val	Glu	Leu
1				5					10					15	
Ala	Glu	Lys	Ala	Met	Lys	Glu	Tyr	Gly	Glu	Asp	Leu	Lys	Ile	Glu	Thr
			20					25					30		
Asn	Lys	Phe	Ala	Ala	Ile	Cys	Thr	His	Leu	Glu	Val	Cys	Phe	Met	Tyr
		35					40					45			
Ser	Asp	Phe	His	Phe	Ile	Asn	Glu	Gln	Gly	Glu	Ser	Ile	Ile	Val	Glu
	50					55					60				
Leu	Asp	Asp	Pro	Asn	Ala	Leu	Leu	Lys	His	Arg	Phe	Glu	Ile	Ile	Glu
65					70					75					80
Gly	Arg	Asp	Arg	Thr	Met	Ala	Trp	Thr	Val	Val	Asn	Ser	Ile	Cys	Asn
				85					90					95	
Thr	Thr	Gly	Ala	Glu	Lys	Pro	Lys	Phe	Leu	Pro	Asp	Leu	Tyr	Asp	Tyr
			100					105					110		
Lys	Glu	Asn	Arg	Phe	Ile	Glu	Ile	Gly	Val	Thr	Arg	Arg	Glu	Val	His
		115					120					125			
Ile	Tyr	Tyr	Leu	Glu	Lys	Ala	Asn	Lys	Ile	Lys	Ser	Glu	Lys	Thr	His
	130					135					140				
Ile	His	Ile	Phe	Ser	Phe	Thr	Gly	Glu	Glu	Met	Ala	Thr	Lys	Ala	Asp
145					150					155					160
Tyr	Thr	Leu	Asp	Glu	Glu	Ser	Arg	Ala	Arg	Ile	Lys	Thr	Arg	Leu	Phe
			165						170					175	
Thr	Ile	Arg	Gln	Glu	Met	Ala	Ser	Arg	Gly	Leu	Trp	Asp	Ser	Phe	His
		180						185					190		
Gln	Ser	Glu	Arg	Gly	Glu	Glu	Thr	Ile	Glu	Glu	Arg	Phe	Glu	Ile	Thr
		195					200					205			
Gly	Thr	Met	Arg	Arg	Leu	Ala	Asp	Gln	Ser	Leu	Pro	Pro	Asn	Phe	Ser
	210					215					220				
Cys	Leu	Glu	Asn	Phe	Arg	Ala	Tyr	Val	Asp	Gly	Phe	Glu	Pro	Asn	Gly
225					230					235					240
Tyr	Ile	Glu	Gly	Lys	Leu	Ser	Gln	Met	Ser	Lys	Glu	Val	Asn	Ala	Lys
			245						250					255	



Ile Glu Pro Phe Leu Lys Thr Thr Pro Arg Pro Ile Arg Leu Pro Asp  
260 265 270

Gly Pro Pro Cys Ser Gln Arg Ser Lys Phe Leu Leu Met Asp Ala Leu  
275 280 285

Lys Leu Ser Ile Glu Asp Pro Ser His Glu Gly Glu Gly Ile Pro Leu  
290 295 300

Tyr Asp Ala Ile Lys Cys Met Arg Thr Phe Phe Gly Trp Lys Glu Pro  
305 310 315 320

Tyr Val Val Lys Pro His Glu Lys Gly Ile Asn Pro Asn Tyr Leu Leu  
325 330 335

Ser Trp Lys Gln Val Leu Ala Glu Leu Gln Asp Ile Glu Asn Glu Glu  
340 345 350

Lys Ile Pro Arg Thr Lys Asn Met Lys Lys Thr Ser Gln Leu Lys Trp  
355 360 365

Ala Leu Gly Glu Asn Met Ala Pro Glu Lys Val Asp Phe Asp Asp Cys  
370 375 380

Arg Asp Val Ser Asp Leu Lys Gln Tyr Asp Ser Asp Glu Pro Glu Leu  
385 390 395 400

Arg Ser Leu Ser Ser Trp Ile Gln Asn Glu Phe Asn Lys Ala Cys Glu  
405 410 415

Leu Thr Asp Ser Ile Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val  
420 425 430

Ala Pro Ile Glu His Ile Ala Ser Met Arg Arg Asn Tyr Phe Thr Ala  
435 440 445

Glu Val Ser His Cys Arg Ala Thr Glu Tyr Ile Met Lys Gly Val Tyr  
450 455 460

Ile Asn Thr Ala Leu Leu Asn Ala Ser Cys Ala Ala Met Asp Asp Phe  
465 470 475 480

Gln Leu Ile Pro Met Ile Ser Lys Cys Arg Thr Lys Glu Gly Arg Arg  
485 490 495

Lys Thr Asn Leu Tyr Gly Phe Ile Ile Lys Gly Arg Ser His Leu Arg  
500 505 510

Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr  
515 520 525

Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu  
530 535 540

Ile Gly Asp Met Leu Leu Arg Ser Ala Ile Gly Gln Val Ser Arg Pro



545		550		555		560									
Met	Phe	Leu	Tyr	Val	Arg	Thr	Asn	Gly	Thr	Ser	Lys	Ile	Lys	Met	Lys
				565					570					575	
Trp	Gly	Met	Glu	Met	Arg	Arg	Cys	Leu	Leu	Gln	Ser	Leu	Gln	Gln	Ile
			580					585					590		
Glu	Ser	Met	Ile	Glu	Ala	Glu	Ser	Ser	Val	Lys	Glu	Lys	Asp	Met	Thr
		595					600					605			
Lys	Glu	Phe	Phe	Glu	Asn	Lys	Ser	Glu	Thr	Trp	Pro	Ile	Gly	Glu	Ser
	610					615					620				
Pro	Lys	Gly	Val	Glu	Glu	Gly	Ser	Ile	Gly	Lys	Val	Cys	Arg	Thr	Leu
	625				630					635					640
Leu	Ala	Lys	Ser	Val	Phe	Asn	Ser	Leu	Tyr	Ala	Ser	Pro	Gln	Leu	Glu
				645					650					655	
Gly	Phe	Ser	Ala	Glu	Ser	Arg	Lys	Leu	Leu	Val	Val	Gln	Ala	Leu	
			660					665				670			
Arg	Asp	Asn	Leu	Glu	Pro	Gly	Thr	Phe	Asp	Leu	Gly	Gly	Leu	Tyr	Glu
		675					680					685			
Ala	Ile	Glu	Glu	Cys	Leu	Ile	Asn	Asp	Pro	Trp	Val	Leu	Leu	Asn	Ala
	690					695					700				
Ser	Trp	Phe	Asn	Ser	Phe	Leu	Thr	His	Ala	Pro	Arg				
705					710					715					

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2341 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: cold adapted "Master Strain" A/AA/6/60 7PI (H2N2)



(vii) IMMEDIATE SOURCE:

(B) CLONE: PB1

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(123, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(486, "u")

(D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(1195, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: mutation

(B) LOCATION: replace(1276, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain; a in  
wt2(3); g in 1988 reported ca vaccine  
strain"  
/citation= ([1][2])



(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(1395, "u")
- (D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(1766, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(2005, "a")
- (D) OTHER INFORMATION: /note= "a in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(2019, "u")
- (D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 25..2295
- (D) OTHER INFORMATION: /product= "polymerase basic 1"  
/gene= "PB1"  
/note= "polymerase basic 1"  
/citation= ([1][2])



(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G
- (B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus
- (C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA
- (G) DATE: 1993
- (K) RELEVANT RESIDUES IN SEQ ID NO:13: FROM 1 TO 2341

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Cox, N J  
Kitame, F  
Kendal, A P  
Maassab, H F  
Naeve, C
- (B) TITLE: Identification of sequence changes in the cold-adapted  
live attenuated influenza vaccine strain
- (C) JOURNAL: Virology
- (D) VOLUME: 167
- (F) PAGES: 554-567
- (G) DATE: 1988
- (K) RELEVANT RESIDUES IN SEQ ID NO:13: FROM 1 TO 2341

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGCGAAAGCA GGCAAACCAU UUGA AUG GAU GUC AAU CCG ACC UUA CUU UUC	51
Met Asp Val Asn Pro Thr Leu Leu Phe	
1 5	
UUG AAA GUU CCA GCG CAA AAU GCC AUA AGU ACU ACA UUC CCU UAU ACU	99
Leu Lys Val Pro Ala Gln Asn Ala Ile Ser Thr Thr Phe Pro Tyr Thr	
10 15 20 25	
GGA GAU CCU CCA UAC AGC CAU GGG ACA GGA ACA GGA UAC ACC AUG GAC	147
Gly Asp Pro Pro Tyr Ser His Gly Thr Gly Thr Gly Tyr Thr Met Asp	
30 35 40	



ACA	GUC	AAC	AGA	ACA	CAU	CAA	UAU	UCA	GAA	AAG	GGG	AAG	UGG	ACA	ACA	195
Thr	Val	Asn	Arg	Thr	His	Gln	Tyr	Ser	Glu	Lys	Gly	Lys	Trp	Thr	Thr	
			45					50					55			
AAC	ACG	GAA	ACU	GGA	GCG	CAC	CAA	CUU	AAC	CCA	AUU	GAU	GGA	CCA	CUA	243
Asn	Thr	Glu	Thr	Gly	Ala	His	Gln	Leu	Asn	Pro	Ile	Asp	Gly	Pro	Leu	
		60					65					70				
CCU	GAG	GAC	AAU	GAA	CCA	AGU	GGA	UAU	GCA	CAA	ACA	GAC	UGC	GUC	CUG	291
Pro	Glu	Asp	Asn	Glu	Pro	Ser	Gly	Tyr	Ala	Gln	Thr	Asp	Cys	Val	Leu	
	75					80					85					
GAA	GCA	AUG	GCU	UUC	CUU	GAA	GAA	UCC	CAC	CCA	GGA	AUC	UUU	GAA	AAC	339
Glu	Ala	Met	Ala	Phe	Leu	Glu	Glu	Ser	His	Pro	Gly	Ile	Phe	Glu	Asn	
90					95					100					105	
UCG	UGU	CUU	GAA	ACG	AUG	GAA	GUU	AUU	CAA	CAA	ACA	AGA	GUG	GAC	AAA	387
Ser	Cys	Leu	Glu	Thr	Met	Glu	Val	Ile	Gln	Gln	Thr	Arg	Val	Asp	Lys	
				110					115					120		
CUG	ACC	CAA	GGU	CGU	CAG	ACC	UAU	GAU	UGG	ACA	UUG	AAC	AGA	AAU	CAG	435
Leu	Thr	Gln	Gly	Arg	Gln	Thr	Tyr	Asp	Trp	Thr	Leu	Asn	Arg	Asn	Gln	
			125					130					135			
CCG	GCU	GCA	ACU	GCG	CUA	GCC	AAC	ACU	AUA	GAG	GUC	UUC	AGA	UCG	AAU	483
Pro	Ala	Ala	Thr	Ala	Leu	Ala	Asn	Thr	Ile	Glu	Val	Phe	Arg	Ser	Asn	
		140					145					150				
GGU	CUG	ACA	GCU	AAU	GAA	UCG	GGA	AGG	CUA	AUA	GAU	UUC	CUC	AAG	GAU	531
Gly	Leu	Thr	Ala	Asn	Glu	Ser	Gly	Arg	Leu	Ile	Asp	Phe	Leu	Lys	Asp	
	155					160					165					
GUG	AUA	GAA	UCA	AUG	GAU	AAA	GAG	GAG	AUG	GAA	AUC	ACA	ACA	CAC	UUC	579
Val	Ile	Glu	Ser	Met	Asp	Lys	Glu	Glu	Met	Glu	Ile	Thr	Thr	His	Phe	
170					175					180					185	
CAA	AGA	AAA	AGA	AGA	GUA	AGA	GAC	AAC	AUG	ACC	AAG	AAA	AUG	GUC	ACA	627
Gln	Arg	Lys	Arg	Arg	Val	Arg	Asp	Asn	Met	Thr	Lys	Lys	Met	Val	Thr	
				190					195					200		
CAA	CGA	ACA	AUA	GGA	AAG	AAG	AAG	CAA	AGA	UUG	AAC	AAG	AGA	AGC	UAU	675
Gln	Arg	Thr	Ile	Gly	Lys	Lys	Lys	Gln	Arg	Leu	Asn	Lys	Arg	Ser	Tyr	
			205					210					215			
CUA	AUA	AGA	GCA	CUG	ACA	UUG	AAC	ACA	AUG	ACU	AAA	GAU	GCA	GAG	AGA	723
Leu	Ile	Arg	Ala	Leu	Thr	Leu	Asn	Thr	Met	Thr	Lys	Asp	Ala	Glu	Arg	
		220					225					230				
GGU	AAA	UUA	AAG	AGA	AGA	GCA	AUU	GCA	ACA	CCC	GGU	AUG	CAG	AUC	AGA	771
Gly	Lys	Leu	Lys	Arg	Arg	Ala	Ile	Ala	Thr	Pro	Gly	Met	Gln	Ile	Arg	
	235					240					245					
GGG	UUC	GUG	UAC	UUU	GUC	GAA	ACA	CUA	GCG	AGA	AGU	AUU	UGU	GAG	AAG	819
Gly	Phe	Val	Tyr	Phe	Val	Glu	Thr	Leu	Ala	Arg	Ser	Ile	Cys	Glu	Lys	
250					255					260					265	



CUU	GAA	CAG	UCU	GGG	CUU	CCG	GUU	GGA	GGU	AAU	GAA	AAG	AAG	GCU	AAA	867
Leu	Glu	Gln	Ser	Gly	Leu	Pro	Val	Gly	Gly	Asn	Glu	Lys	Lys	Ala	Lys	
				270					275					280		
CUG	GCA	AAU	GUU	GUG	CGA	AAA	AUG	AUG	ACU	AAU	UCA	CAA	GAC	ACA	GAG	915
Leu	Ala	Asn	Val	Val	Arg	Lys	Met	Met	Thr	Asn	Ser	Gln	Asp	Thr	Glu	
			285					290					295			
CUC	UCU	UUC	ACA	AUU	ACU	GGA	GAC	AAU	ACC	AAA	UGG	AAU	GAG	AAU	CAA	963
Leu	Ser	Phe	Thr	Ile	Thr	Gly	Asp	Asn	Thr	Lys	Trp	Asn	Glu	Asn	Gln	
		300					305					310				
AAU	CCU	CGG	AUG	UUC	CUG	GCG	AUG	AUA	ACA	UAC	AUC	ACA	AGA	AAU	CAA	1011
Asn	Pro	Arg	Met	Phe	Leu	Ala	Met	Ile	Thr	Tyr	Ile	Thr	Arg	Asn	Gln	
	315					320					325					
CCU	GAA	UGG	UUU	AGA	AAC	GUC	CUG	AGC	AUC	GCA	CCU	AUA	AUG	UUC	UCA	1059
Pro	Glu	Trp	Phe	Arg	Asn	Val	Leu	Ser	Ile	Ala	Pro	Ile	Met	Phe	Ser	
330					335					340					345	
AAU	AAA	AUG	GCA	AGA	CUA	GGG	AAA	GGA	UAC	AUG	UUC	AAA	AGC	AAG	AGC	1107
Asn	Lys	Met	Ala	Arg	Leu	Gly	Lys	Gly	Tyr	Met	Phe	Lys	Ser	Lys	Ser	
				350					355					360		
AUG	AAG	CUC	CGA	ACA	CAA	AUA	CCA	GCA	GAA	AUG	CUA	GCA	AGU	AUU	GAC	1155
Met	Lys	Leu	Arg	Thr	Gln	Ile	Pro	Ala	Glu	Met	Leu	Ala	Ser	Ile	Asp	
			365					370					375			
CUG	AAA	UAC	UUU	AAU	GAA	UCA	ACA	AGA	AAG	AAA	AUC	GAG	GAA	AUA	AGG	1203
Leu	Lys	Tyr	Phe	Asn	Glu	Ser	Thr	Arg	Lys	Lys	Ile	Glu	Glu	Ile	Arg	
		380					385					390				
CCU	CUC	CUA	AUA	GAU	GGC	ACA	GUC	UCA	UUG	AGU	CCU	GGA	AUG	AUG	AUG	1251
Pro	Leu	Leu	Ile	Asp	Gly	Thr	Val	Ser	Leu	Ser	Pro	Gly	Met	Met	Met	
	395					400					405					
GGC	AUG	UUC	AAC	AUG	CUA	AGU	ACA	GUC	UUA	GGA	GUC	UCA	AUC	CUG	AAU	1299
Gly	Met	Phe	Asn	Met	Leu	Ser	Thr	Val	Leu	Gly	Val	Ser	Ile	Leu	Asn	
410					415					420					425	
CUU	GGA	CAA	AAG	AAG	UAC	ACC	AAA	ACA	ACA	UAC	UGG	UGG	GAC	GGA	CUC	1347
Leu	Gly	Gln	Lys	Lys	Tyr	Thr	Lys	Thr	Thr	Tyr	Trp	Trp	Asp	Gly	Leu	
				430					435					440		
CAA	UCC	UCU	GAU	GAC	UUC	GCC	CUC	AUA	GUG	AAU	GCA	CCA	AAU	CAU	GAU	1395
Gln	Ser	Ser	Asp	Asp	Phe	Ala	Leu	Ile	Val	Asn	Ala	Pro	Asn	His	Asp	
			445					450					455			
GGA	AUA	CAA	GCA	GGG	GUG	GAU	AGA	UUC	UAC	AGA	ACC	UGC	AAG	CUA	GUC	1443
Gly	Ile	Gln	Ala	Gly	Val	Asp	Arg	Phe	Tyr	Arg	Thr	Cys	Lys	Leu	Val	
		460					465					470				
GGA	AUC	AAU	AUG	AGC	AAA	AAG	AAG	UCC	UAC	AUA	AAU	AGG	ACA	GGG	ACA	1491
Gly	Ile	Asn	Met	Ser	Lys	Lys	Lys	Ser	Tyr	Ile	Asn	Arg	Thr	Gly	Thr	
	475					480					485					



UUU Phe 490	GAA Glu	UUC Phe	ACA Thr	AGC Ser	UUU Phe 495	UUC Phe	UAU Tyr	CGC Arg	UAU Tyr	GGA Gly 500	UUU Phe	GUA Val	GCC Ala	AAU Asn	UUU Phe 505	1539
AGC Ser	AUG Met	GAG Glu	CUG Leu	CCC Pro 510	AGC Ser	UUU Phe	GGA Gly	GUG Val	UCU Ser 515	GGA Gly	AUU Ile	AAU Asn	GAA Glu	UCG Ser 520	GCU Ala	1587
GAU Asp	AUG Met	AGC Ser	AUU Ile 525	GGG Gly	GUA Val	ACA Thr	GUG Val	AUA Ile 530	AAG Lys	AAC Asn	AAC Asn	AUG Met	AUA Ile 535	AAC Asn	AAU Asn	1635
GAC Asp	CUU Leu	GGG Gly 540	CCA Pro	GCA Ala	ACA Thr	GCC Ala	CAA Gln 545	CUG Leu	GCU Ala	CUU Leu	CAA Gln	CUA Leu 550	UUC Phe	AUC Ile	AAA Lys	1683
GAC Asp 555	UAC Tyr	AGA Arg	UAU Tyr	ACG Thr	UAC Tyr	CGG Arg 560	UGC Cys	CAC His	AGA Arg	GGA Gly	GAC Asp 565	ACA Thr	CAA Gln	AUU Ile	CAG Gln	1731
ACA Thr 570	AGG Arg	AGA Arg	UCA Ser	UUC Phe	GAG Glu 575	CUA Leu	AAG Lys	AAG Lys	CUG Leu	UGG Trp 580	GGG Gly	CAA Gln	ACC Thr	CGC Arg	UCA Ser 585	1779
AAG Lys	GCA Ala	GGA Gly	CUU Leu	UUG Leu 590	GUU Val	UCG Ser	GAU Asp	GGA Gly	GGA Gly 595	CCA Pro	AAC Asn	UUA Leu	UAC Tyr	AAU Asn 600	AUC Ile	1827
CGG Arg	AAU Asn	CUC Leu	CAC His 605	AUU Ile	CCA Pro	GAA Glu	GUC Val	UGC Cys 610	UUG Leu	AAG Lys	UGG Trp	GAG Glu	CUA Leu 615	AUG Met	GAU Asp	1875
GAA Glu	GAC Asp	UAU Tyr 620	CAG Gln	GGG Gly	AGG Arg	CUU Leu	UGU Cys 625	AAU Asn	CCC Pro	CUG Leu	AAU Asn	CCA Pro 630	UUU Phe	GUC Val	AGU Ser	1923
CAU His 635	AAG Lys	GAG Glu	AUU Ile	GAG Glu	UCU Ser	GUA Val 640	AAC Asn	AAU Asn	GCU Ala	GUG Val	GUA Val 645	AUG Met	CCA Pro	GCU Ala	CAC His	1971
GGU Gly 650	CCA Pro	GCC Ala	AAG Lys	AGC Ser	AUG Met 655	GAA Glu	UAU Tyr	GAU Asp	GCU Ala	GUU Val 660	ACU Thr	ACU Thr	ACA Thr	CAC His	UCU Ser 665	2019
UGG Trp	AUC Ile	CCU Pro	AAG Lys	AGG Arg 670	AAC Asn	CGC Arg	UCC Ser	AUU Ile	CUC Leu 675	AAC Asn	ACA Thr	AGC Ser	CAA Gln	AGG Arg 680	GGA Gly	2067
AUU Ile	CUU Leu	GAA Glu	GAU Asp 685	GAA Glu	CAG Gln	AUG Met	UAU Tyr	CAG Gln 690	AAG Lys	UGU Cys	UGC Cys	AAU Asn	CUA Leu 695	UUC Phe	GAG Glu	2115
AAA Lys	UUC Phe	UUC Phe	CCU Pro	AGC Ser	AGU Ser	UCG Ser	UAC Tyr 705	AGG Arg	AGA Arg	CCA Pro	GUU Val	GGA Gly 710	AUU Ile	UCC Ser	AGC Ser	2163



AUG GUG GAG GCC AUG GUG UCU AGG GCC CGG AUU GAU GCA CGG AUU GAC	2211
Met Val Glu Ala Met Val Ser Arg Ala Arg Ile Asp Ala Arg Ile Asp	
715 720 725	
UUC GAG UCU GGA CGG AUU AAG AAA GAG GAG UUC GCU GAG AUC AUG AAG	2259
Phe Glu Ser Gly Arg Ile Lys Lys Glu Glu Phe Ala Glu Ile Met Lys	
730 735 740 745	
AUC UGU UCC ACC AUU GAA GAG CUC AGA CGG CAA AAA UAGUGAAUUU	2305
Ile Cys Ser Thr Ile Glu Glu Leu Arg Arg Gln Lys	
750 755	
AGCUUGUCCU UCAUGAAAAA AUGCCUUGUU UCUACU	2341

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 757 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met	Asp	Val	Asn	Pro	Thr	Leu	Leu	Phe	Leu	Lys	Val	Pro	Ala	Gln	Asn
1				5					10					15	
Ala	Ile	Ser	Thr	Thr	Phe	Pro	Tyr	Thr	Gly	Asp	Pro	Pro	Tyr	Ser	His
			20					25					30		
Gly	Thr	Gly	Thr	Gly	Tyr	Thr	Met	Asp	Thr	Val	Asn	Arg	Thr	His	Gln
		35					40					45			
Tyr	Ser	Glu	Lys	Gly	Lys	Trp	Thr	Thr	Asn	Thr	Glu	Thr	Gly	Ala	His
	50					55					60				
Gln	Leu	Asn	Pro	Ile	Asp	Gly	Pro	Leu	Pro	Glu	Asp	Asn	Glu	Pro	Ser
65					70					75				80	
Gly	Tyr	Ala	Gln	Thr	Asp	Cys	Val	Leu	Glu	Ala	Met	Ala	Phe	Leu	Glu
				85					90					95	
Glu	Ser	His	Pro	Gly	Ile	Phe	Glu	Asn	Ser	Cys	Leu	Glu	Thr	Met	Glu
			100					105					110		
Val	Ile	Gln	Gln	Thr	Arg	Val	Asp	Lys	Leu	Thr	Gln	Gly	Arg	Gln	Thr
		115					120					125			



Tyr Asp Trp Thr Leu Asn Arg Asn Gln Pro Ala Ala Thr Ala Leu Ala  
 130 135 140  
 Asn Thr Ile Glu Val Phe Arg Ser Asn Gly Leu Thr Ala Asn Glu Ser  
 145 150 155 160  
 Gly Arg Leu Ile Asp Phe Leu Lys Asp Val Ile Glu Ser Met Asp Lys  
 165 170 175  
 Glu Glu Met Glu Ile Thr Thr His Phe Gln Arg Lys Arg Arg Val Arg  
 180 185 190  
 Asp Asn Met Thr Lys Lys Met Val Thr Gln Arg Thr Ile Gly Lys Lys  
 195 200 205  
 Lys Gln Arg Leu Asn Lys Arg Ser Tyr Leu Ile Arg Ala Leu Thr Leu  
 210 215 220  
 Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala  
 225 230 235 240  
 Ile Ala Thr Pro Gly Met Gln Ile Arg Gly Phe Val Tyr Phe Val Glu  
 245 250 255  
 Thr Leu Ala Arg Ser Ile Cys Glu Lys Leu Glu Gln Ser Gly Leu Pro  
 260 265 270  
 Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ala Asn Val Val Arg Lys  
 275 280 285  
 Met Met Thr Asn Ser Gln Asp Thr Glu Leu Ser Phe Thr Ile Thr Gly  
 290 295 300  
 Asp Asn Thr Lys Trp Asn Glu Asn Gln Asn Pro Arg Met Phe Leu Ala  
 305 310 315 320  
 Met Ile Thr Tyr Ile Thr Arg Asn Gln Pro Glu Trp Phe Arg Asn Val  
 325 330 335  
 Leu Ser Ile Ala Pro Ile Met Phe Ser Asn Lys Met Ala Arg Leu Gly  
 340 345 350  
 Lys Gly Tyr Met Phe Lys Ser Lys Ser Met Lys Leu Arg Thr Gln Ile  
 355 360 365  
 Pro Ala Glu Met Leu Ala Ser Ile Asp Leu Lys Tyr Phe Asn Glu Ser  
 370 375 380  
 Thr Arg Lys Lys Ile Glu Glu Ile Arg Pro Leu Leu Ile Asp Gly Thr  
 385 390 395 400  
 Val Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu Ser  
 405 410 415



Thr Val Leu Gly Val Ser Ile Leu Asn Leu Gly Gln Lys Lys Tyr Thr  
 420 425 430  
 Lys Thr Thr Tyr Trp Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala  
 435 440 445  
 Leu Ile Val Asn Ala Pro Asn His Asp Gly Ile Gln Ala Gly Val Asp  
 450 455 460  
 Arg Phe Tyr Arg Thr Cys Lys Leu Val Gly Ile Asn Met Ser Lys Lys  
 465 470 475 480  
 Lys Ser Tyr Ile Asn Arg Thr Gly Thr Phe Glu Phe Thr Ser Phe Phe  
 485 490 495  
 Tyr Arg Tyr Gly Phe Val Ala Asn Phe Ser Met Glu Leu Pro Ser Phe  
 500 505 510  
 Gly Val Ser Gly Ile Asn Glu Ser Ala Asp Met Ser Ile Gly Val Thr  
 515 520 525  
 Val Ile Lys Asn Asn Met Ile Asn Asn Asp Leu Gly Pro Ala Thr Ala  
 530 535 540  
 Gln Leu Ala Leu Gln Leu Phe Ile Lys Asp Tyr Arg Tyr Thr Tyr Arg  
 545 550 555 560  
 Cys His Arg Gly Asp Thr Gln Ile Gln Thr Arg Arg Ser Phe Glu Leu  
 565 570 575  
 Lys Lys Leu Trp Gly Gln Thr Arg Ser Lys Ala Gly Leu Leu Val Ser  
 580 585 590  
 Asp Gly Gly Pro Asn Leu Tyr Asn Ile Arg Asn Leu His Ile Pro Glu  
 595 600 605  
 Val Cys Leu Lys Trp Glu Leu Met Asp Glu Asp Tyr Gln Gly Arg Leu  
 610 615 620  
 Cys Asn Pro Leu Asn Pro Phe Val Ser His Lys Glu Ile Glu Ser Val  
 625 630 635 640  
 Asn Asn Ala Val Val Met Pro Ala His Gly Pro Ala Lys Ser Met Glu  
 645 650 655  
 Tyr Asp Ala Val Thr Thr Thr His Ser Trp Ile Pro Lys Arg Asn Arg  
 660 665 670  
 Ser Ile Leu Asn Thr Ser Gln Arg Gly Ile Leu Glu Asp Glu Gln Met  
 675 680 685  
 Tyr Gln Lys Cys Cys Asn Leu Phe Glu Lys Phe Phe Pro Ser Ser Ser  
 690 695 700



Tyr Arg Arg Pro Val Gly Ile Ser Ser Met Val Glu Ala Met Val Ser  
705 710 715 720  
Arg Ala Arg Ile Asp Ala Arg Ile Asp Phe Glu Ser Gly Arg Ile Lys  
725 730 735  
Lys Glu Glu Phe Ala Glu Ile Met Lys Ile Cys Ser Thr Ile Glu Glu  
740 745 750  
Leu Arg Arg Gln Lys  
755

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2341 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: cold-adapted "Master Strain" A/Ann Arbor/6/60 7PI (H2N2)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: PB2

(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(141, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain; a in wt2(3); g in 1988 reported ca vaccine strain"  
/citation= ([1][2])



(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(426, "c")
- (D) OTHER INFORMATION: /note= "c in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(714, "u")
- (D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3); c in 1988 reported ca vaccine  
strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(821, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(963, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); a in 1988 reported ca vaccine  
strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(1182, "u")
- (D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])



(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(1212, "u")
- (D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(1353, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(1923, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3)"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(1933, "c")
- (D) OTHER INFORMATION: /note= "c in ca "master" strain; u in  
wt2(3); u in 1988 reported ca vaccine  
strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 28..2304
- (D) OTHER INFORMATION: /product= "polymerase basic 2"  
/gene= "PB2"  
/note= "polymerase basic 2"  
/citation= ([1][2])



Thr	Phe	Gly	Pro	Val	His	Phe	Arg	Asn	Gln	Val	Lys	Ile	Arg	Arg	Arg
130						135					140				
Val	Asp	Ile	Asn	Pro	Gly	His	Ala	Asp	Leu	Ser	Ala	Lys	Glu	Ala	Gln
145					150					155					160
Asp	Val	Ile	Met	Glu	Val	Val	Phe	Pro	Asn	Glu	Val	Gly	Ala	Arg	Ile
				165					170					175	
Leu	Thr	Ser	Glu	Ser	Gln	Leu	Thr	Ile	Thr	Lys	Glu	Lys	Lys	Glu	Glu
			180					185					190		
Leu	Gln	Asp	Cys	Lys	Ile	Ser	Pro	Leu	Met	Val	Ala	Tyr	Met	Leu	Glu
		195					200					205			
Arg	Glu	Leu	Val	Arg	Lys	Thr	Arg	Phe	Leu	Pro	Val	Ala	Gly	Gly	Thr
	210					215					220				
Ser	Ser	Val	Tyr	Ile	Glu	Val	Leu	His	Leu	Thr	Gln	Gly	Thr	Cys	Trp
225					230					235					240
Glu	Gln	Met	Tyr	Thr	Pro	Gly	Gly	Glu	Val	Arg	Asn	Asp	Asp	Val	Asp
				245					250					255	
Gln	Ser	Leu	Ile	Ile	Ala	Ala	Arg	Ser	Ile	Val	Arg	Arg	Ala	Ala	Val
			260					265					270		
Ser	Ala	Asp	Pro	Leu	Ala	Ser	Leu	Leu	Glu	Met	Cys	His	Ser	Thr	Gln
		275					280					285			
Ile	Gly	Gly	Thr	Arg	Met	Val	Asp	Ile	Leu	Arg	Gln	Asn	Pro	Thr	Glu
	290					295					300				
Glu	Gln	Ala	Val	Glu	Ile	Cys	Lys	Ala	Ala	Met	Gly	Leu	Arg	Ile	Ser
305					310					315					320
Ser	Ser	Phe	Ser	Phe	Gly	Gly	Phe	Thr	Phe	Lys	Arg	Thr	Ser	Gly	Ser
				325					330					335	
Ser	Val	Lys	Arg	Glu	Glu	Glu	Val	Leu	Thr	Gly	Asn	Leu	Gln	Thr	Leu
			340					345					350		
Lys	Ile	Arg	Val	His	Glu	Gly	Tyr	Glu	Glu	Phe	Thr	Met	Val	Gly	Lys
		355					360					365			
Arg	Ala	Thr	Ala	Ile	Leu	Arg	Lys	Ala	Thr	Arg	Arg	Leu	Ile	Gln	Leu
		370				375					380				
Ile	Val	Ser	Gly	Arg	Asp	Glu	Gln	Ser	Ile	Ala	Glu	Ala	Ile	Ile	Val
385					390					395					400
Ala	Met	Val	Phe	Ser	Gln	Glu	Asp	Cys	Met	Ile	Lys	Ala	Val	Arg	Gly
				405					410					415	



Asp Leu Asn Phe Val Asn Arg Ala Asn Gln Arg Leu Asn Pro Met His  
 420 425 430  
 Gln Leu Leu Arg His Phe Gln Lys Asp Ala Lys Val Leu Phe Gln Asn  
 435 440 445  
 Trp Gly Ile Glu His Ile Asp Asn Val Met Gly Met Ile Gly Val Leu  
 450 455 460  
 Pro Asp Met Thr Pro Ser Thr Glu Met Ser Met Arg Gly Val Arg Val  
 465 470 475 480  
 Ser Lys Met Gly Val Asp Glu Tyr Ser Ser Ala Glu Arg Val Val Val  
 485 490 495  
 Ser Ile Asp Arg Phe Leu Arg Val Arg Asp Gln Arg Gly Asn Val Leu  
 500 505 510  
 Leu Ser Pro Glu Glu Val Ser Glu Thr Gln Gly Thr Glu Lys Leu Thr  
 515 520 525  
 Ile Thr Tyr Ser Ser Ser Met Met Trp Glu Ile Asn Gly Pro Glu Ser  
 530 535 540  
 Val Leu Val Asn Thr Tyr Gln Trp Ile Ile Arg Asn Trp Glu Thr Val  
 545 550 555 560  
 Lys Ile Gln Trp Ser Gln Asn Pro Thr Met Leu Tyr Asn Lys Met Glu  
 565 570 575  
 Phe Glu Pro Phe Gln Ser Leu Val Pro Lys Ala Ile Arg Gly Gln Tyr  
 580 585 590  
 Ser Gly Phe Val Arg Thr Leu Phe Gln Gln Met Arg Asp Val Leu Gly  
 595 600 605  
 Thr Phe Asp Thr Thr Gln Ile Ile Lys Leu Leu Pro Phe Ala Ala Ala  
 610 615 620  
 Pro Pro Lys Gln Ser Arg Met Gln Phe Ser Ser Leu Thr Val Asn Val  
 625 630 635 640  
 Arg Gly Ser Gly Met Arg Ile Leu Val Arg Gly Asn Ser Pro Ile Phe  
 645 650 655  
 Asn Tyr Asn Lys Thr Thr Lys Arg Leu Thr Ile Leu Gly Lys Asp Ala  
 660 665 670  
 Gly Thr Leu Thr Glu Asp Pro Asp Glu Gly Thr Ser Gly Val Glu Ser  
 675 680 685  
 Ala Val Leu Arg Gly Phe Leu Ile Leu Gly Lys Glu Asp Arg Arg Tyr  
 690 695 700



Gly Pro Ala Leu Ser Ile Asn Glu Leu Ser Asn Leu Ala Lys Gly Glu  
705 710 715 720  
Lys Ala Asn Val Leu Ile Gly Gln Gly Asp Val Val Leu Val Met Lys  
725 730 735  
Arg Lys Arg Asn Ser Ser Ile Leu Thr Asp Ser Gln Thr Ala Thr Lys  
740 745 750  
Arg Ile Arg Met Ala Ile Asn  
755

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1773 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: cold-adapted "Master Strain" A/Ann Arbor/6/60 7PI (H2N2)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: HA

(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(144, "u")
- (D) OTHER INFORMATION: /gene= "HA"  
/note= "u in ca "master" strain; a in  
w2(3)"  
/citation= ([1])



(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(455, "a")
- (D) OTHER INFORMATION: /gene= "HA"  
/note= "a in ca "master" strain; g in  
wt2(3)"  
/citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(729, "c")
- (D) OTHER INFORMATION: /gene= "HA"  
/note= "c in ca "master" strain; a in  
wt2(3)"  
/citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 44..1729
- (D) OTHER INFORMATION: /product= "hemagglutinin"  
/gene= "HA"  
/note= "hemagglutinin protein"  
/citation= ([1])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G
- (B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus
- (C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA
- (G) DATE: 1993
- (K) RELEVANT RESIDUES IN SEQ ID NO:17: FROM 1 TO 1773



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGCAAAAGCA	GGGUUAUAC	CAUAGACAAC	CAAAAGCAAA	ACA	AUG	GCC	AUC	AUU		55						
					Met	Ala	Ile	Ile								
					1											
UAU	CUC	AUU	CUC	CUG	UUC	ACA	GCA	GUG	AGA	GGG	GAC	AAG	AUA	UGC	AUU	103
Tyr	Leu	Ile	Leu	Leu	Phe	Thr	Ala	Val	Arg	Gly	Asp	Lys	Ile	Cys	Ile	
5					10					15					20	
GGA	UAC	CAU	GCC	AAU	AAU	UCC	ACA	GAG	ACG	GUC	GAC	ACA	AUU	CUA	GAG	151
Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Glu	Thr	Val	Asp	Thr	Ile	Leu	Glu	
				25				30						35		
CGG	AAC	GUC	ACU	GUG	ACU	CAU	GCC	AAG	GAC	AUU	CUU	GAG	AAG	ACC	CAU	199
Arg	Asn	Val	Thr	Val	Thr	His	Ala	Lys	Asp	Ile	Leu	Glu	Lys	Thr	His	
			40					45					50			
AAC	GGA	AAG	UUA	UGC	AAA	CUA	AAC	GGA	AUC	CCU	CCA	CUU	GAA	CUA	GGG	247
Asn	Gly	Lys	Leu	Cys	Lys	Leu	Asn	Gly	Ile	Pro	Pro	Leu	Glu	Leu	Gly	
		55					60					65				
GAC	UGU	AGC	AUU	GCC	GGA	UGG	CUC	CUU	GGA	AAU	CCA	GAA	UGU	GAU	AGG	295
Asp	Cys	Ser	Ile	Ala	Gly	Trp	Leu	Leu	Gly	Asn	Pro	Glu	Cys	Asp	Arg	
	70					75					80					
CUU	CUA	AGU	GUG	CCA	GAA	UGG	UCC	UAU	AUA	AUG	GAG	AAA	GAA	AAC	CCG	343
Leu	Leu	Ser	Val	Pro	Glu	Trp	Ser	Tyr	Ile	Met	Glu	Lys	Glu	Asn	Pro	
85					90					95					100	
AGA	AAC	GGU	UUG	UGU	UAU	CCA	GGC	AAC	UUC	AAU	GAU	UAU	GAA	GAA	UUG	391
Arg	Asn	Gly	Leu	Cys	Tyr	Pro	Gly	Asn	Phe	Asn	Asp	Tyr	Glu	Glu	Leu	
				105				110						115		
AAA	CAU	CUC	CUC	AGC	AGC	GUG	AAA	CAU	UUC	GAG	AAA	GUA	AAG	AUU	CUG	439
Lys	His	Leu	Leu	Ser	Ser	Val	Lys	His	Phe	Glu	Lys	Val	Lys	Ile	Leu	
			120					125					130			
CCC	AAA	GAU	AGA	UGG	ACA	CAG	CAU	ACA	ACA	ACU	GGA	GGU	UCA	CAG	GCC	487
Pro	Lys	Asp	Arg	Trp	Thr	Gln	His	Thr	Thr	Thr	Gly	Gly	Ser	Gln	Ala	
		135					140					145				
UGC	GCG	GUG	UCU	GGU	AAU	CCA	UCA	UUC	UUC	AGG	AAC	AUG	GUC	UGG	CUG	535
Cys	Ala	Val	Ser	Gly	Asn	Pro	Ser	Phe	Phe	Arg	Asn	Met	Val	Trp	Leu	
	150					155					160					
ACA	GAG	GAA	GGA	UCA	AAU	UAU	CCG	GUU	GCC	AAA	GGA	UCG	UAC	AAC	AAU	583
Thr	Glu	Glu	Gly	Ser	Asn	Tyr	Pro	Val	Ala	Lys	Gly	Ser	Tyr	Asn	Asn	
165					170					175					180	
ACA	AGC	GGA	GAA	CAA	AUG	CUA	AUA	AUU	UGG	GGG	GUG	CAC	CAU	CCC	AUU	631
Thr	Ser	Gly	Glu	Gln	Met	Leu	Ile	Ile	Trp	Gly	Val	His	His	Pro	Ile	
				185					190					195		



GAU Asp	GAG Glu	ACA Thr	GAA Glu 200	CAA Gln	AGA Arg	ACA Thr	UUG Leu	UAC Tyr 205	CAG Gln	AAU Asn	GUG Val	GGA Gly	ACC Thr 210	UAU Tyr	GUU Val	679
UCC Ser	GUA Val	GGC Gly 215	ACA Thr	UCA Ser	ACA Thr	UUG Leu	AAC Asn 220	AAA Lys	AGG Arg	UCA Ser	ACC Thr	CCA Pro 225	GAA Glu	AUA Ile	GCA Ala	727
ACA Thr 230	AGG Arg	CCU Pro	AAA Lys	GUG Val	AAU Asn	GGA Gly 235	CUA Leu	GGA Gly	AGU Ser	AGA Arg	AUG Met 240	GAA Glu	UUC Phe	UCU Ser	UGG Trp	775
ACC Thr 245	CUC Leu	UUG Leu	GAU Asp	AUG Met	UGG Trp 250	GAC Asp	ACC Thr	AUA Ile	AAU Asn 255	UUU Phe	GAG Glu	AGU Ser	ACU Thr	GGU Gly	AAU Asn 260	823
CUA Leu	AUU Ile	GCA Ala	CCA Pro	GAG Glu 265	UAU Tyr	GGA Gly	UUC Phe	AAA Lys 270	AUA Ile	UCG Ser	AAA Lys	AGA Arg	GGU Gly	AGU Ser 275	UCU Ser	871
GGG Gly	AUC Ile	AUG Met	AAA Lys 280	ACA Thr	GAA Glu	GGA Gly	ACA Thr	CUU Leu 285	GAG Glu	AAC Asn	UGU Cys	GAG Glu	ACC Thr 290	AAA Lys	UGC Cys	919
CAA Gln	ACU Thr	CCU Pro 295	UUG Leu	GGA Gly	GCA Ala	AUA Ile	AAU Asn 300	ACA Thr	ACA Thr	UUG Leu	CCU Pro	UUU Phe 305	CAC His	AAU Asn	GUC Val	967
CAC His 310	CCA Pro	CUG Leu	ACA Thr	AUA Ile	GGU Gly	GAG Glu 315	UGC Cys	CCC Pro	AAA Lys	UAU Tyr	GUA Val 320	AAA Lys	UCG Ser	GAG Glu	AAG Lys	1015
UUG Leu 325	GUC Val	UUA Leu	GCA Ala	ACA Thr	GGA Gly 330	CUA Leu	AGG Arg	AAU Asn	GUU Val	CCC Pro 335	CAG Gln	AUU Ile	GAA Glu	UCA Ser	AGA Arg 340	1063
GGA Gly	UUG Leu	UUU Phe	GGG Gly 345	GCA Ala	AUA Ile	GCU Ala	GGU Gly	UUU Phe 350	AUA Ile	GAA Glu	GGA Gly	GGA Gly	UGG Trp	CAA Gln 355	GGA Gly	1111
AUG Met	GUU Val	GAU Asp	GGU Gly 360	UGG Trp	UAU Tyr	GGA Gly	UAC Tyr	CAU His 365	CAC His	AGC Ser	AAU Asn	GAC Asp	CAG Gln 370	GGA Gly	UCA Ser	1159
GGG Gly	UAU Tyr	GCA Ala 375	GCA Ala	GAC Asp	AAA Lys	GAA Glu	UCC Ser 380	ACU Thr	CAA Gln	AAG Lys	GCA Ala	UUU Phe 385	GAU Asp	GGA Gly	AUC Ile	1207
ACC Thr	AAC Asn 390	AAG Lys	GUA Val	AAU Asn	UCU Ser	GUG Val 395	AUU Ile	GAA Glu	AAG Lys	AUA Ile	AAC Asn 400	ACC Thr	CAA Gln	UUU Phe	GAA Glu	1255
GCU Ala 405	GUU Val	GGG Gly	AAA Lys	GAA Glu	UUC Phe 410	AGU Ser	AAC Asn	UUA Leu	GAG Glu	AGA Arg 415	AGA Arg	CUG Leu	GAG Glu	AAC Asn	UUG Leu 420	1303



AAC AAA AAG AUG GAA GAC GGG UUU CUA GAU GUG UGG ACA UAC AAU GCU Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala 425 430 435	1351
GAG CUU CUA GUU CUG AUG GAA AAU GAG AGG ACA CUU GAC UUU CAU GAU Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu Asp Phe His Asp 440 445 450	1399
UCU AAU GUC AAG AAU CUG UAU GAU AAA GUC AGA AUG CAG CUG AGG GAC Ser Asn Val Lys Asn Leu Tyr Asp Lys Val Arg Met Gln Leu Arg Asp 455 460 465	1447
AAC GUC AAA GAA CUA GGA AAU GGA UGU UUU GAA UUU UAU CAC AAA UGU Asn Val Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys 470 475 480	1495
GAU GAU GAA UGC AUG AAU AGU GUG AAA AAC GGG ACA UAU GAU UAU CCC Asp Asp Glu Cys Met Asn Ser Val Lys Asn Gly Thr Tyr Asp Tyr Pro 485 490 495 500	1543
AAG UAU GAA GAA GAG UCU AAA CUA AAU AGA AAU GAA AUU AAA GGG GUA Lys Tyr Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu Ile Lys Gly Val 505 510 515	1591
AAA UUG AGC AGC AUG GGG GUU UGU CGG AUC CUU GCC AUU UAU GCU ACA Lys Leu Ser Ser Met Gly Val Cys Arg Ile Leu Ala Ile Tyr Ala Thr 520 525 530	1639
GUA GCA GGU UCU CUG UCA CUG GCA AUC AUG AUG GCU GGG AUC UCU UUC Val Ala Gly Ser Leu Ser Leu Ala Ile Met Met Ala Gly Ile Ser Phe 535 540 545	1687
UGG AUG UGC UCC AAC GGG UCU CUG CAG UGC AGG AUC UGC AUA Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile 550 555 560	1729
UGAUUAUAAG UCAUUUUUAUA AUUAAAAACA CCCUUGUUUC UACU	1773

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 562 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Asp  
1 5 10 15  
Lys Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Thr Val Asp  
20 25 30  
Thr Ile Leu Glu Arg Asn Val Thr Val Thr His Ala Lys Asp Ile Leu  
35 40 45  
Glu Lys Thr His Asn Gly Lys Leu Cys Lys Leu Asn Gly Ile Pro Pro  
50 55 60  
Leu Glu Leu Gly Asp Cys Ser Ile Ala Gly Trp Leu Leu Gly Asn Pro  
65 70 75 80  
Glu Cys Asp Arg Leu Leu Ser Val Pro Glu Trp Ser Tyr Ile Met Glu  
85 90 95  
Lys Glu Asn Pro Arg Asn Gly Leu Cys Tyr Pro Gly Asn Phe Asn Asp  
100 105 110  
Tyr Glu Glu Leu Lys His Leu Leu Ser Ser Val Lys His Phe Glu Lys  
115 120 125  
Val Lys Ile Leu Pro Lys Asp Arg Trp Thr Gln His Thr Thr Thr Gly  
130 135 140  
Gly Ser Gln Ala Cys Ala Val Ser Gly Asn Pro Ser Phe Phe Arg Asn  
145 150 155 160  
Met Val Trp Leu Thr Glu Glu Gly Ser Asn Tyr Pro Val Ala Lys Gly  
165 170 175  
Ser Tyr Asn Asn Thr Ser Gly Glu Gln Met Leu Ile Ile Trp Gly Val  
180 185 190  
His His Pro Ile Asp Glu Thr Glu Gln Arg Thr Leu Tyr Gln Asn Val  
195 200 205  
Gly Thr Tyr Val Ser Val Gly Thr Ser Thr Leu Asn Lys Arg Ser Thr  
210 215 220  
Pro Glu Ile Ala Thr Arg Pro Lys Val Asn Gly Leu Gly Ser Arg Met  
225 230 235 240  
Glu Phe Ser Trp Thr Leu Leu Asp Met Trp Asp Thr Ile Asn Phe Glu  
245 250 255  
Ser Thr Gly Asn Leu Ile Ala Pro Glu Tyr Gly Phe Lys Ile Ser Lys  
260 265 270  
Arg Gly Ser Ser Gly Ile Met Lys Thr Glu Gly Thr Leu Glu Asn Cys  
275 280 285



Glu Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro  
 290 295 300  
 Phe His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val  
 305 310 315 320  
 Lys Ser Glu Lys Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro Gln  
 325 330 335  
 Ile Glu Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly  
 340 345 350  
 Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His Ser Asn  
 355 360 365  
 Asp Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala  
 370 375 380  
 Phe Asp Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu Lys Ile Asn  
 385 390 395 400  
 Thr Gln Phe Glu Ala Val Gly Lys Glu Phe Ser Asn Leu Glu Arg Arg  
 405 410 415  
 Leu Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp  
 420 425 430  
 Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu  
 435 440 445  
 Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val Arg Met  
 450 455 460  
 Gln Leu Arg Asp Asn Val Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe  
 465 470 475 480  
 Tyr His Lys Cys Asp Asp Glu Cys Met Asn Ser Val Lys Asn Gly Thr  
 485 490 495  
 Tyr Asp Tyr Pro Lys Tyr Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu  
 500 505 510  
 Ile Lys Gly Val Lys Leu Ser Ser Met Gly Val Cys Arg Ile Leu Ala  
 515 520 525  
 Ile Tyr Ala Thr Val Ala Gly Ser Leu Ser Leu Ala Ile Met Met Ala  
 530 535 540  
 Gly Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile  
 545 550 555 560  
 Cys Ile



(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1467 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: cold-adapted "Master Strain" A/Ann Arbor/6/60 7PI (H2N2)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: NA

(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(394, "u")
- (D) OTHER INFORMATION: /product= "Neuraminidase"  
/gene= "NA"  
/note= "u in ca "master" strain; c in  
wt2(3)"  
/citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(604, "u")
- (D) OTHER INFORMATION: /product= "Neuraminidase"  
/gene= "NA"  
/note= "u in ca "master" strain; a in  
wt2(3)"  
/citation= ([1])



(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 20..1426
- (D) OTHER INFORMATION: /product= "neuraminidase"  
/gene= "NA"  
/note= "neuraminidase protein"  
/citation= ([1])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G
- (B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) Influenza Virus
- (C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA
- (G) DATE: 1993
- (K) RELEVANT RESIDUES IN SEQ ID NO:19: FROM 1 TO 1467

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AGCAAAAGCA GGAGUGAAA AUG AAU CCA AAU CAA AAG ACA AUA ACA AUU GGC	52
Met Asn Pro Asn Gln Lys Thr Ile Thr Ile Gly	
1 5 10	
UCU GUC UCU CUC ACC AUC GCA ACA GUA UGC UUC CUC AUG CAG AUU GCC	100
Ser Val Ser Leu Thr Ile Ala Thr Val Cys Phe Leu Met Gln Ile Ala	
15 20 25	
AUC CUG GCA ACU ACU GUG ACA UUG CAC CUU AAG CAA CAU GAG UGC GAC	148
Ile Leu Ala Thr Thr Val Thr Leu His Leu Lys Gln His Glu Cys Asp	
30 35 40	
UCC CCC GCG AGC AAC CAA GUA AUG CCA UGU GAA CCA AUA AUA AUA GAA	196
Ser Pro Ala Ser Asn Gln Val Met Pro Cys Glu Pro Ile Ile Ile Glu	
45 50 55	
AGG AAC AUA ACA GAG AUA GUG UAU UUG AAU AAC ACC ACC AUA GAG AAA	244
Arg Asn Ile Thr Glu Ile Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys	
60 65 70 75	
GAG AUU UGC CCC GAA GUA GUG GGA UAC AGA AAU UGG UCA AAG CCG CAA	292
Glu Ile Cys Pro Glu Val Val Gly Tyr Arg Asn Trp Ser Lys Pro Gln	
80 85 90	



UGU	CAA	AUU	ACA	GGA	UUU	GCA	CCU	UUU	UCU	AAG	GAC	AAU	UCA	AUC	CGG	340
Cys	Gln	Ile	Thr	Gly	Phe	Ala	Pro	Phe	Ser	Lys	Asp	Asn	Ser	Ile	Arg	
			95					100					105			
CUU	UCU	GCU	GGU	GGG	GAC	AUU	UGG	GUG	ACG	AGA	GAA	CCU	UAU	GUG	UCA	388
Leu	Ser	Ala	Gly	Gly	Asp	Ile	Trp	Val	Thr	Arg	Glu	Pro	Tyr	Val	Ser	
		110					115					120				
UGC	GAU	CCU	GGC	AAG	UGU	UAU	CAA	UUU	GCA	CUC	GGG	CAG	GGG	ACC	ACA	436
Cys	Asp	Pro	Gly	Lys	Cys	Tyr	Gln	Phe	Ala	Leu	Gly	Gln	Gly	Thr	Thr	
	125					130					135					
CUA	GAC	AAC	AAA	CAU	UCA	AAU	GGC	ACA	AUA	CAU	GAU	AGA	AUC	CCU	CAU	484
Leu	Asp	Asn	Lys	His	Ser	Asn	Gly	Thr	Ile	His	Asp	Arg	Ile	Pro	His	
140					145				150						155	
CGA	ACC	CUA	UUA	AUG	AAU	GAG	UUG	GGU	GUU	CCA	UUU	CAU	UUA	GGA	ACC	532
Arg	Thr	Leu	Leu	Met	Asn	Glu	Leu	Gly	Val	Pro	Phe	His	Leu	Gly	Thr	
				160					165					170		
AAA	CAA	GUG	UGU	GCA	GCA	UGG	UCC	AGC	UCA	AGU	UGU	CAC	GAU	GGA	AAA	580
Lys	Gln	Val	Cys	Ala	Ala	Trp	Ser	Ser	Ser	Ser	Cys	His	Asp	Gly	Lys	
			175					180					185			
GCA	UGG	UUG	CAU	GUU	UGU	GUC	ACU	GGG	GAU	GAU	AGA	AAU	GCA	ACU	GCU	628
Ala	Trp	Leu	His	Val	Cys	Val	Thr	Gly	Asp	Asp	Arg	Asn	Ala	Thr	Ala	
		190					195					200				
AGC	UUC	AUU	UAU	GAC	GGG	AAG	CUU	GUG	GAC	AGU	AUU	GGU	UCA	UGG	UCU	676
Ser	Phe	Ile	Tyr	Asp	Gly	Lys	Leu	Val	Asp	Ser	Ile	Gly	Ser	Trp	Ser	
	205					210					215					
CAA	AAU	GUC	CUC	AGG	ACC	CAG	GAG	UCG	GAA	UGC	GUC	UGU	AUC	AAU	GGG	724
Gln	Asn	Val	Leu	Arg	Thr	Gln	Glu	Ser	Glu	Cys	Val	Cys	Ile	Asn	Gly	
220					225					230					235	
ACU	UGC	ACA	GUA	GUA	AUG	ACU	GAU	GGA	AGU	GCA	UCA	GGA	AGA	GCU	GAU	772
Thr	Cys	Thr	Val	Val	Met	Thr	Asp	Gly	Ser	Ala	Ser	Gly	Arg	Ala	Asp	
				240					245					250		
ACU	AGA	AUA	CUA	UUC	AUU	AAA	GAG	GGG	AAA	AUU	GUC	CAU	AUU	GGC	CCA	820
Thr	Arg	Ile	Leu	Phe	Ile	Lys	Glu	Gly	Lys	Ile	Val	His	Ile	Gly	Pro	
			255					260					265			
UUG	UCA	GGA	AGU	GCU	CAG	CAU	GUA	GAG	GAG	UGU	UCU	UGU	UAC	CCU	CGA	868
Leu	Ser	Gly	Ser	Ala	Gln	His	Val	Glu	Glu	Cys	Ser	Cys	Tyr	Pro	Arg	
		270					275					280				
UAU	CCU	GAC	GUC	AGA	UGU	AUC	UGC	AGA	GAC	AAC	UGG	AAA	GGC	UCU	AAU	916
Tyr	Pro	Asp	Val	Arg	Cys	Ile	Cys	Arg	Asp	Asn	Trp	Lys	Gly	Ser	Asn	
	285					290					295					
AGG	CCC	GUU	AUA	GAC	AUA	AAU	AUG	GAA	GAU	UAU	AGC	AUU	GAU	UCC	AGU	964
Arg	Pro	Val	Ile	Asp	Ile	Asn	Met	Glu	Asp	Tyr	Ser	Ile	Asp	Ser	Ser	
300					305					310					315	



UAU GUG UGC UCA GGG CUU GUU GGC GAC ACA CCC AGG AAC GAC GAC AGC Tyr Val Cys Ser Gly Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser 320 325 330	1012
UCU AGC AAU AGC AAU UGC AGG GAU CCU AAC AAU GAG AGA GGG AAU CCA Ser Ser Asn Ser Asn Cys Arg Asp Pro Asn Asn Glu Arg Gly Asn Pro 335 340 345	1060
GGA GUG AAA GGC UGG GCC UUU GAC AAU GGA GAU GAU GUA UGG AUG GGA Gly Val Lys Gly Trp Ala Phe Asp Asn Gly Asp Asp Val Trp Met Gly 350 355 360	1108
AGA ACA AUC AGC AAA GAU UUA CGC UCA GGU UAU GAA ACU UUC AAA GUC Arg Thr Ile Ser Lys Asp Leu Arg Ser Gly Tyr Glu Thr Phe Lys Val 365 370 375	1156
AUU GGU GGU UGG UCC ACA CCU AAU UCC AAA UCG CAG GUC AAU AGA CAG Ile Gly Gly Trp Ser Thr Pro Asn Ser Lys Ser Gln Val Asn Arg Gln 380 385 390 395	1204
GUC AUA GUU GAC AAC AAU AAU UGG UCU GGU UAC UCU GGU AUU UUC UCU Val Ile Val Asp Asn Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser 400 405 410	1252
GUU GAG GGC AAA AGC UGC AUC AAU AGG UGC UUU UAU GUG GAG UUG AUA Val Glu Gly Lys Ser Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile 415 420 425	1300
AGG GGA AGG CCA CAG GAG ACU AGA GUA UGG UGG ACC UCA AAC AGU AUU Arg Gly Arg Pro Gln Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile 430 435 440	1348
GUU GUA UUU UGU GGC ACU UCA GGU ACU UAU GGA ACA GGC UCA UGG CCU Val Val Phe Cys Gly Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro 445 450 455	1396
GAU GGG GCG AAC AUC AAU UUC AUG CCU AUA UAACGUUUCG CAAUUUUAGA Asp Gly Ala Asn Ile Asn Phe Met Pro Ile 460 465	1446
AAAAAACUCC UUGUUUCUAC U	1467

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 469 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear



(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Asn Pro Asn Gln Lys Thr Ile Thr Ile Gly Ser Val Ser Leu Thr  
1 5 10 15  
Ile Ala Thr Val Cys Phe Leu Met Gln Ile Ala Ile Leu Ala Thr Thr  
20 25 30  
Val Thr Leu His Leu Lys Gln His Glu Cys Asp Ser Pro Ala Ser Asn  
35 40 45  
Gln Val Met Pro Cys Glu Pro Ile Ile Ile Glu Arg Asn Ile Thr Glu  
50 55 60  
Ile Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys Glu Ile Cys Pro Glu  
65 70 75 80  
Val Val Gly Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly  
85 90 95  
Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly  
100 105 110  
Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Gly Lys  
115 120 125  
Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Asp Asn Lys His  
130 135 140  
Ser Asn Gly Thr Ile His Asp Arg Ile Pro His Arg Thr Leu Leu Met  
145 150 155 160  
Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Ala  
165 170 175  
Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val  
180 185 190  
Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Phe Ile Tyr Asp  
195 200 205  
Gly Lys Leu Val Asp Ser Ile Gly Ser Trp Ser Gln Asn Val Leu Arg  
210 215 220  
Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Thr Val Val  
225 230 235 240  
Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe  
245 250 255  
Ile Lys Glu Gly Lys Ile Val His Ile Gly Pro Leu Ser Gly Ser Ala  
260 265 270



Gln His Val Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Asp Val Arg  
275 280 285

Cys Ile Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Ile Asp  
290 295 300

Ile Asn Met Glu Asp Tyr Ser Ile Asp Ser Ser Tyr Val Cys Ser Gly  
305 310 315 320

Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser Ser Ser Asn Ser Asn  
325 330 335

Cys Arg Asp Pro Asn Asn Glu Arg Gly Asn Pro Gly Val Lys Gly Trp  
340 345 350

Ala Phe Asp Asn Gly Asp Asp Val Trp Met Gly Arg Thr Ile Ser Lys  
355 360 365

Asp Leu Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Gly Gly Trp Ser  
370 375 380

Thr Pro Asn Ser Lys Ser Gln Val Asn Arg Gln Val Ile Val Asp Asn  
385 390 395 400

Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser  
405 410 415

Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Gln  
420 425 430

Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly  
435 440 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile  
450 455 460

Asn Phe Met Pro Ile  
465

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 890 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)



(vi) ORIGINAL SOURCE:

(A) ORGANISM: Influenza virus

(B) STRAIN: wild type A/Ann Arbor/6/60 (H2N2) Egg Passage 2(3)

(vii) IMMEDIATE SOURCE:

(B) CLONE: NS

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION: 27..56

(D) OTHER INFORMATION: /product= "nonstructural protein NS2"  
/gene= "NS"  
/note= "nonstructural protein NS2"  
/citation= ([1][2])

(ix) FEATURE:.

(A) NAME/KEY: conflict

(B) LOCATION: replace(483, "a")

(D) OTHER INFORMATION: /note= "a in ca "master" strain and in  
wt2(3); g in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION: 529..861

(D) OTHER INFORMATION: /product= "nonstructural protein NS2"  
/gene= "NS"  
/note= "nonstructural protein NS2"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(813, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in



wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(27..56, 529..861)
- (D) OTHER INFORMATION: /product= "nonstructural protein NS2"  
/gene= "NS"  
/note= "nonstructural protein NS2"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 27..677
- (D) OTHER INFORMATION: /product= "nonstructural protein NS1"  
/gene= "NS"  
/note= "nonstructural protein NS1"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G
- (B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus
- (C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA
- (G) DATE: 1993
- (K) RELEVANT RESIDUES IN SEQ ID NO:21: FROM 1 TO 890

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Cox, N J  
Kitame, F  
Kendal, A P  
Maassab, H F  
Naeve, C







(B) TITLE: Identification of sequence changes in the cold-adapted  
live attenuated influenza vaccine strain, A/Ann  
Arbor/6/60(H2N2)

(C) JOURNAL: Virology

(D) VOLUME: 167

(F) PAGES: 554-567

(G) DATE: 1988

(K) RELEVANT RESIDUES IN SEQ ID NO:21: FROM 1 TO 890

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AGCAAAAGCA GGGUGACAAA GACAUA AUG GAU CCU AAC ACU GUG UCA AGC UUU	53
Met Asp Pro Asn Thr Val Ser Ser Phe	
1 5	
CAG GUA GAU UGC UUC CUU UGG CAU GUC CGC AAA CAA GUU GCA GAC CAA	101
Gln Val Asp Cys Phe Leu Trp His Val Arg Lys Gln Val Ala Asp Gln	
10 15 20 25	
GAA CUA GGU GAU GCC CCA UUC CUU GAU CGG CUU CGC CGA GAU CAG AAG	149
Glu Leu Gly Asp Ala Pro Phe Leu Asp Arg Leu Arg Arg Asp Gln Lys	
30 35 40	
UCC CUA AGG GGA AGA GGC AGU ACU CUC GGU CUG AAC AUC GAA ACA GCC	197
Ser Leu Arg Gly Arg Gly Ser Thr Leu Gly Leu Asn Ile Glu Thr Ala	
45 50 55	
ACC CGU GUU GGA AAG CAG AUA GUG GAG AGG AUU CUG AAG GAA GAA UCC	245
Thr Arg Val Gly Lys Gln Ile Val Glu Arg Ile Leu Lys Glu Glu Ser	
60 65 70	
GAU GAG GCA CUU AAA AUG ACC AUG GCC UCC GCA CCU GCU UCG CGA UAC	293
Asp Glu Ala Leu Lys Met Thr Met Ala Ser Ala Pro Ala Ser Arg Tyr	
75 80 85	
CUA ACU GAC AUG ACU AUU GAG GAA AUG UCA AGG GAC UGG UUC AUG CUA	341
Leu Thr Asp Met Thr Ile Glu Glu Met Ser Arg Asp Trp Phe Met Leu	
90 95 100 105	
AUG CCC AAG CAG AAA GUG GCA GGC CCU CUU UGU AUC AGA AUG GAC CAG	389
Met Pro Lys Gln Lys Val Ala Gly Pro Leu Cys Ile Arg Met Asp Gln	
110 115 120	
GCA AUC AUG GAU AAG AAC AUC AUA UUG AAA GCG AAU UUC AGU GUG AUU	437
Ala Ile Met Asp Lys Asn Ile Ile Leu Lys Ala Asn Phe Ser Val Ile	
125 130 135	



UUU GAC CGG CUA GAG ACC CUA AUA UUA CUA AGG GCU UUC ACC GAA ACG Phe Asp Arg Leu Glu Thr Leu Ile Leu Leu Arg Ala Phe Thr Glu Thr 140 145 150	485
GGA GCA AUU GUU GGC GAA AUU UCA CCA UUG CCU UCU CUU CCA GGA CAU Gly Ala Ile Val Gly Glu Ile Ser Pro Leu Pro Ser Leu Pro Gly His 155 160 165	533
ACU AAU GAG GAU GUC AAA AAU GCA AUU GGG GUC CUC AUC GGA GGA CUU Thr Asn Glu Asp Val Lys Asn Ala Ile Gly Val Leu Ile Gly Gly Leu 170 175 180 185	581
GAA UGG AAU GAU AAC ACA GUU CGA GUC UCU AAA ACU CUA CAG AGA UUC Glu Trp Asn Asp Asn Thr Val Arg Val Ser Lys Thr Leu Gln Arg Phe 190 195 200	629
GCU UGG AGA AGC AGU GAU GAG AAU GGG AGA CCU CCA CUC ACU CCA AAA Ala Trp Arg Ser Ser Asp Glu Asn Gly Arg Pro Pro Leu Thr Pro Lys 205 210 215	677
UAGAAACGGA AAAUGGCGAG AACAAUUAGG UCAAAAGUUC GAAGAAAUAA GAUGGCUGAU	737
UGAAGAAGUG AGACACAAAU UGAAGAUAAAC AGAGAAUAGU UUUGAGCAAA UAACAUUUAU	797
GCAAGCCUUA CAGCUGCUAU UUGAAGUGGA ACAAGAGAUU AGAACUUUCU CGUUUCAGCU	857
UAUUUAAUGA UAAAAACAC CCUUGUUUCU ACU	890

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 217 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met	Asp	Pro	Asn	Thr	Val	Ser	Ser	Phe	Gln	Val	Asp	Cys	Phe	Leu	Trp
1				5					10					15	
His	Val	Arg	Lys	Gln	Val	Ala	Asp	Gln	Glu	Leu	Gly	Asp	Ala	Pro	Phe
			20					25					30		
Leu	Asp	Arg	Leu	Arg	Arg	Asp	Gln	Lys	Ser	Leu	Arg	Gly	Arg	Gly	Ser
		35					40					45			



Thr Leu Gly Leu Asn Ile Glu Thr Ala Thr Arg Val Gly Lys Gln Ile  
50 55 60  
Val Glu Arg Ile Leu Lys Glu Glu Ser Asp Glu Ala Leu Lys Met Thr  
65 70 75 80  
Met Ala Ser Ala Pro Ala Ser Arg Tyr Leu Thr Asp Met Thr Ile Glu  
85 90 95  
Glu Met Ser Arg Asp Trp Phe Met Leu Met Pro Lys Gln Lys Val Ala  
100 105 110  
Gly Pro Leu Cys Ile Arg Met Asp Gln Ala Ile Met Asp Lys Asn Ile  
115 120 125  
Ile Leu Lys Ala Asn Phe Ser Val Ile Phe Asp Arg Leu Glu Thr Leu  
130 135 140  
Ile Leu Leu Arg Ala Phe Thr Glu Thr Gly Ala Ile Val Gly Glu Ile  
145 150 155 160  
Ser Pro Leu Pro Ser Leu Pro Gly His Thr Asn Glu Asp Val Lys Asn  
165 170 175  
Ala Ile Gly Val Leu Ile Gly Gly Leu Glu Trp Asn Asp Asn Thr Val  
180 185 190  
Arg Val Ser Lys Thr Leu Gln Arg Phe Ala Trp Arg Ser Ser Asp Glu  
195 200 205  
Asn Gly Arg Pro Pro Leu Thr Pro Lys  
210 215

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 418 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 27..389



(D) OTHER INFORMATION: /product= "Nonstructural protein 2"  
/gene= "NS2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AGCAAAAGCA GGGUGACAAA GACAU	AUG GAU CCU AAC ACU GUG UCA AGC UUU	53
	Met Asp Pro Asn Thr Val Ser Ser Phe	
	1 5	
CAG GAC AUA CUA AUG AGG AUG UCA AAA AUG CAA UUG GGG UCC UCA UCG		101
Gln Asp Ile Leu Met Arg Met Ser Lys Met Gln Leu Gly Ser Ser Ser		
10 15 20 25		
GAG GAC UUG AAU GGA AUG AUA ACA CAG UUC GAG UCU CUA AAA CUC UAC		149
Glu Asp Leu Asn Gly Met Ile Thr Gln Phe Glu Ser Leu Lys Leu Tyr		
30 35 40		
AGA GAU UCG CUU GGA GAA GCA GUG AUG AGA AUG GGA GAC CUC CAC UCA		197
Arg Asp Ser Leu Gly Glu Ala Val Met Arg Met Gly Asp Leu His Ser		
45 50 55		
CUC CAA AAU AGA AAC GGA AAA UGG CGA GAA CAA UUA GGU CAA AAG UUC		245
Leu Gln Asn Arg Asn Gly Lys Trp Arg Glu Gln Leu Gly Gln Lys Phe		
60 65 70		
GAA GAA AUA AGA UGG CUG AUU GAA GAA GUG AGA CAC AAA UUG AAG AUA		293
Glu Glu Ile Arg Trp Leu Ile Glu Glu Val Arg His Lys Leu Lys Ile		
75 80 85		
ACA GAG AAU AGU UUU GAG CAA AUA ACA UUU AUG CAA GCC UUA CAG CUG		341
Thr Glu Asn Ser Phe Glu Gln Ile Thr Phe Met Gln Ala Leu Gln Leu		
90 95 100 105		
CUA UUU GAA GUG GAA CAA GAG AUA AGA ACU UUC UCG UUU CAG CUU AUU		389
Leu Phe Glu Val Glu Gln Glu Ile Arg Thr Phe Ser Phe Gln Leu Ile		
110 115 120		
UAAUGAUAAA AAACACCCUU GUUUCUACU		418

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 121 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Asp Pro Asn Thr Val Ser Ser Phe Gln Asp Ile Leu Met Arg Met  
1 5 10 15  
Ser Lys Met Gln Leu Gly Ser Ser Ser Glu Asp Leu Asn Gly Met Ile  
20 25 30  
Thr Gln Phe Glu Ser Leu Lys Leu Tyr Arg Asp Ser Leu Gly Glu Ala  
35 40 45  
Val Met Arg Met Gly Asp Leu His Ser Leu Gln Asn Arg Asn Gly Lys  
50 55 60  
Trp Arg Glu Gln Leu Gly Gln Lys Phe Glu Glu Ile Arg Trp Leu Ile  
65 70 75 80  
Glu Glu Val Arg His Lys Leu Lys Ile Thr Glu Asn Ser Phe Glu Gln  
85 90 95  
Ile Thr Phe Met Gln Ala Leu Gln Leu Leu Phe Glu Val Glu Gln Glu  
100 105 110  
Ile Arg Thr Phe Ser Phe Gln Leu Ile  
115 120

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1027 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: wild type A/Ann Arbor/6/60 (H2N2) Egg Passage 2(3)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: M



(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 26..51
- (D) OTHER INFORMATION: /product= "matrix protein M2"  
/gene= "M"  
/note= "matrix protein M2"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 740..1004
- (D) OTHER INFORMATION: /product= "matrix protein M2"  
/gene= "M"  
/note= "matrix protein M2"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(969, "u")
- (D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3); g in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(26..51, 740..1004)
- (D) OTHER INFORMATION: /product= "matrix protein M2"  
/gene= "M"  
/note= "matrix protein M2"  
/citation= ([1][2])



(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 26..781
- (D) OTHER INFORMATION: /product= "matrix protein M1"  
/gene= "M"  
/note= "matrix protein M1"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G
- (B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus
- (C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA
- (G) DATE: 1993
- (K) RELEVANT RESIDUES IN SEQ ID NO:25: FROM 1 TO 1027

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Cox, N J  
Kitame, F  
Kendal, A P  
Maassab, H F  
Naeve, C
- (B) TITLE: Identification of sequence changes in the cold-adapted  
live attenuated influenza vaccine strain, A/Ann  
Arbor/6/60(H2N2)
- (C) JOURNAL: Virology
- (D) VOLUME: 167
- (F) PAGES: 554-557
- (G) DATE: 1988
- (K) RELEVANT RESIDUES IN SEQ ID NO:25: FROM 1 TO 1027.



AGCAAAAGCA	GGUAGAUUU	GAAAG	AUG Met 1	AGU Ser	CUU Leu	CUA Leu	ACC Thr 5	GAG Glu	GUC Val	GAA Glu	ACG Thr		52			
UAC Tyr 10	GUU Val	CUC Leu	UCU Ser	AUC Ile	AUC Ile 15	CCG Pro	UCA Ser	GGC Gly	CCC Pro	CUC Leu 20	AAA Lys	GCC Ala	GAG Glu	AUC Ile	GCA Ala 25	100
CAG Gln	AGA Arg	CUU Leu	GAA Glu	GAU Asp 30	GUC Val	UUU Phe	GCU Ala	GGG Gly	AAA Lys 35	AAC Asn	ACC Thr	GAU Asp	CUU Leu	GAG Glu 40	GCU Ala	148
CUC Leu	AUG Met	GAA Glu	UGG Trp 45	CUA Leu	AAG Lys	ACA Thr	AGA Arg	CCA Pro 50	AUC Ile	CUG Leu	UCA Ser	CCU Pro	CUG Leu 55	ACU Thr	AAG Lys	196
GGG Gly	AUU Ile	UUG Leu 60	GGA Gly	UUU Phe	GUA Val	UUC Phe	ACG Thr 65	CUC Leu	ACC Thr	GUG Val	CCC Pro	AGU Ser 70	GAG Glu	CGA Arg	GGA Gly	244
CUG Leu	CAG Gln 75	CGU Arg	AGA Arg	CGC Arg	UUU Phe	GUC Val 80	CAA Gln	AAU Asn	GCC Ala	CUC Leu	AAU Asn 85	GGG Gly	AAU Asn	GGG Gly	GAU Asp	292
CCA Pro 90	AAU Asn	AAC Asn	AUG Met	GAC Asp	AGA Arg 95	GCA Ala	GUU Val	AAA Lys	CUG Leu	UAU Tyr 100	AGA Arg	AAG Lys	CUU Leu	AAG Lys	AGG Arg 105	340
GAG Glu	AUA Ile	ACA Thr	UUC Phe	CAU His 110	GGG Gly	GCC Ala	AAA Lys	GAA Glu	AUA Ile 115	GCG Ala	CUC Leu	AGU Ser	UAU Tyr	UCU Ser 120	GCU Ala	388
GGU Gly	GCA Ala	CUU Leu	GCC Ala 125	AGU Ser	UGU Cys	AUG Met	GGC Gly	CUC Leu 130	AUA Ile	UAC Tyr	AAC Asn	AGG Arg	AUG Met 135	GGG Gly	GCU Ala	436
GUG Val	ACC Thr	ACU Thr 140	GAA Glu	GUG Val	GUC Val	UUA Leu	GGC Gly 145	CUG Leu	GUA Val	UGU Cys	GCA Ala	ACC Thr 150	UGU Cys	GAA Glu	CAG Gln	484
AUU Ile	GCU Ala 155	GAC Asp	UCC Ser	CAG Gln	CAU His	AGG Arg 160	UCU Ser	CAU His	AGG Arg	CAA Gln	AUG Met 165	GUG Val	ACA Thr	ACA Thr	ACC Thr	532
AAU Asn 170	CCA Pro	CUA Leu	AUA Ile	AGA Arg	CAU His 175	GAG Glu	AAC Asn	AGA Arg	AUG Met	GUU Val 180	CUG Leu	GCC Ala	AGC Ser	ACU Thr	ACA Thr 185	580
GCU Ala	AAG Lys	GCU Ala	AUG Met	GAG Glu 190	CAA Gln	AUG Met	GCU Ala	GGA Gly	UCG Ser 195	AGU Ser	GAG Glu	CAA Gln	GCA Ala	GCA Ala 200	GAG Glu	628



GCC AUG GAG GUU GCU AGU CAG GCC AGG CAA AUG GUG CAG GCA AUG AGA Ala Met Glu Val Ala Ser Gln Ala Arg Gln Met Val Gln Ala Met Arg 205 210 215	676
GUU AUU GGG ACU CAU CCU AGC UCC AGU GCU GGU CUA AAA AAU GAU CUU Val Ile Gly Thr His Pro Ser Ser Ser Ala Gly Leu Lys Asn Asp Leu 220 225 230	724
CUU GAA AAU UUG CAG GCC UAU CAG AAA CGA AUG GGG GUG CAG AUG CAA Leu Glu Asn Leu Gln Ala Tyr Gln Lys Arg Met Gly Val Gln Met Gln 235 240 245	772
CGA UUC AAG UGACCCUCUU GUUGUUGCCG CGAGUAUCAU UGGGAUCUUG Arg Phe Lys 250	821
CACUUGAUAU UGUGGAUUCU UGAUCAUCUU UUUUUCAAU GCAUUUAUCG CUUCUUUAAA	881
CACGGUCUGA AAAGAGGGCC UUCUACGGAA GGAGUACCAG AGUCUAUGAG GGAAGAAUAV	941
CGAAAGGAAC AGCAGAGUGC UGUGGAUUCU GACGAUAGUC AUUUUGUCAG CAUAGAGCUG	1001
GAGUAAAAAA CUACCUUGUU UCUACU	1027

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 252 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met	Ser	Leu	Leu	Thr	Glu	Val	Glu	Thr	Tyr	Val	Leu	Ser	Ile	Ile	Pro
1				5					10					15	
Ser	Gly	Pro	Leu	Lys	Ala	Glu	Ile	Ala	Gln	Arg	Leu	Glu	Asp	Val	Phe
			20					25					30		
Ala	Gly	Lys	Asn	Thr	Asp	Leu	Glu	Ala	Leu	Met	Glu	Trp	Leu	Lys	Thr
		35					40					45			
Arg	Pro	Ile	Leu	Ser	Pro	Leu	Thr	Lys	Gly	Ile	Leu	Gly	Phe	Val	Phe
	50					55					60				
Thr	Leu	Thr	Val	Pro	Ser	Glu	Arg	Gly	Leu	Gln	Arg	Arg	Arg	Phe	Val



65		70		75		80									
Gln	Asn	Ala	Leu	Asn	Gly	Asn	Gly	Asp	Pro	Asn	Asn	Met	Asp	Arg	Ala
			85						90					95	
Val	Lys	Leu	Tyr	Arg	Lys	Leu	Lys	Arg	Glu	Ile	Thr	Phe	His	Gly	Ala
			100					105					110		
Lys	Glu	Ile	Ala	Leu	Ser	Tyr	Ser	Ala	Gly	Ala	Leu	Ala	Ser	Cys	Met
		115					120					125			
Gly	Leu	Ile	Tyr	Asn	Arg	Met	Gly	Ala	Val	Thr	Thr	Glu	Val	Val	Leu
	130					135					140				
Gly	Leu	Val	Cys	Ala	Thr	Cys	Glu	Gln	Ile	Ala	Asp	Ser	Gln	His	Arg
145					150					155					160
Ser	His	Arg	Gln	Met	Val	Thr	Thr	Thr	Asn	Pro	Leu	Ile	Arg	His	Glu
			165						170					175	
Asn	Arg	Met	Val	Leu	Ala	Ser	Thr	Thr	Ala	Lys	Ala	Met	Glu	Gln	Met
			180					185					190		
Ala	Gly	Ser	Ser	Glu	Gln	Ala	Ala	Glu	Ala	Met	Glu	Val	Ala	Ser	Gln
		195					200					205			
Ala	Arg	Gln	Met	Val	Gln	Ala	Met	Arg	Val	Ile	Gly	Thr	His	Pro	Ser
	210					215					220				
Ser	Ser	Ala	Gly	Leu	Lys	Asn	Asp	Leu	Leu	Glu	Asn	Leu	Gln	Ala	Tyr
225					230					235					240
Gln	Lys	Arg	Met	Gly	Val	Gln	Met	Gln	Arg	Phe	Lys				
				245					250						

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 339 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)



(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 26..316

(D) OTHER INFORMATION: /product= "Matrix M2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AGCAAAAGCA	GGUAGAUUU	GAAAG	AUG	AGU	CUU	CUA	ACC	GAG	GUC	GAA	ACG		52			
			Met	Ser	Leu	Leu	Thr	Glu	Val	Glu	Thr					
			1				5									
CCU	AUC	AGA	AAC	GAA	UGG	GGG	UGC	AGA	UGC	AAC	GAU	UCA	AGU	GAC	CCU	100
Pro	Ile	Arg	Asn	Glu	Trp	Gly	Cys	Arg	Cys	Asn	Asp	Ser	Ser	Asp	Pro	
10					15					20					25	
CUU	GUU	GUU	GCC	GCG	AGU	AUC	AUU	GGG	AUC	UUG	CAC	UUG	AUA	UUG	UGG	148
Leu	Val	Val	Ala	Ala	Ser	Ile	Ile	Gly	Ile	Leu	His	Leu	Ile	Leu	Trp	
			30					35					40			
AUU	CUU	GAU	CAU	CUU	UUU	UUC	AAA	UGC	AUU	UAU	CGC	UUC	UUU	AAA	CAC	196
Ile	Leu	Asp	His	Leu	Phe	Phe	Lys	Cys	Ile	Tyr	Arg	Phe	Phe	Lys	His	
			45					50					55			
GGU	CUG	AAA	AGA	GGG	CCU	UCU	ACG	GAA	GGA	GUA	CCA	GAG	UCU	AUG	AGG	244
Gly	Leu	Lys	Arg	Gly	Pro	Ser	Thr	Glu	Gly	Val	Pro	Glu	Ser	Met	Arg	
		60					65					70				
GAA	GAA	UAU	CGA	AAG	GAA	CAG	CAG	AGU	GCU	GUG	GAU	UCU	GAC	GAU	AGU	292
Glu	Glu	Tyr	Arg	Lys	Glu	Gln	Gln	Ser	Ala	Val	Asp	Ser	Asp	Asp	Ser	
	75					80					85					
CAU	UUU	GUC	AGC	AUA	GAG	CUG	GAG	UAAAAACUA	CCUUGUUUCU	ACU						339
His	Phe	Val	Ser	Ile	Glu	Leu	Glu									
90					95											

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Ser Leu Leu Thr Glu Val Glu Thr Pro Ile Arg Asn Glu Trp Gly  
1 5 10 15  
Cys Arg Cys Asn Asp Ser Ser Asp Pro Leu Val Val Ala Ala Ser Ile  
20 25 30  
Ile Gly Ile Leu His Leu Ile Leu Trp Ile Leu Asp His Leu Phe Phe  
35 40 45  
Lys Cys Ile Tyr Arg Phe Phe Lys His Gly Leu Lys Arg Gly Pro Ser  
50 55 60  
Thr Glu Gly Val Pro Glu Ser Met Arg Glu Glu Tyr Arg Lys Glu Gln  
65 70 75 80  
Gln Ser Ala Val Asp Ser Asp Asp Ser His Phe Val Ser Ile Glu Leu  
85 90 95  
Glu

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2341 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: wild type A/Ann Arbor/6/60 (H2N2) egg passage 2(3)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: PB2



(ix) FEATURE:

(A) NAME/KEY: mutation

(B) LOCATION: replace(141, "a")

(D) OTHER INFORMATION: /note= "g in ca "master" strain; a in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(426, "c")

(D) OTHER INFORMATION: /note= "c in ca "master" strain and in  
wt2(3); u in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(714, "u")

(D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3); c in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(821, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])



(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(963, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(1182, "u")

(D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(1212, "u")

(D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3); c in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(1353, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); u in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])



(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(1923, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(1933, "u")
- (D) OTHER INFORMATION: /note= "c in ca "master" strain; u in  
wt2(3); u in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 28..2304
- (D) OTHER INFORMATION: /product= "polymerase basic 2"  
/gene= "PB2"  
/note= "polymerase basic 2"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G
- (B) TITLE: Molecular and biological changes in the cold  
adapted master strain A/AA/6/60 (H2N2) influenza virus
- (C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA
- (G) DATE: 1993
- (K) RELEVANT RESIDUES IN SEQ ID NO:29: FROM 1 TO 2341



(x) PUBLICATION INFORMATION:

(A) AUTHORS: Cox, N J  
Kitame, F  
Kendal, A P  
Maassab, H F  
Naeve, C

(B) TITLE: Identification of sequence changes in the cold-adapted  
live attenuated influenza vaccine strain, A/Ann  
Arbor/6/60(H2N2)

(C) JOURNAL: Virology

(D) VOLUME: 167

(F) PAGES: 554-567

(G) DATE: 1988

(K) RELEVANT RESIDUES IN SEQ ID NO:29: FROM 1 TO 2341

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AGCGAAAGCA	GGUCAAUU	AUUCAAU	AUG GAA AGA AUA AAA GAA CUA CGG	51
			Met Glu Arg Ile Lys Glu Leu Arg	
			1 5	
AAU CUG AUG UCG CAG UCU CGC ACU CGC GAG AUA CUA ACA AAA ACC ACA	99			
Asn Leu Met Ser Gln Ser Arg Thr Arg Glu Ile Leu Thr Lys Thr Thr				
10 15 20				
GUG GAC CAU AUG GCC AUA AUU AAG AAG UAC ACA UCA GGG AGA CAG GAA	147			
Val Asp His Met Ala Ile Ile Lys Lys Tyr Thr Ser Gly Arg Gln Glu				
25 30 35 40				
AAG AAC CCG UCA CUU AGG AUG AAA UGG AUG AUG GCA AUG AAA UAU CCG	195			
Lys Asn Pro Ser Leu Arg Met Lys Trp Met Met Ala Met Lys Tyr Pro				
45 50 55				
AUU ACA GCC GAC AAG AGG AUA ACA GAA AUG AUU CCU GAG AGA AAU GAG	243			
Ile Thr Ala Asp Lys Arg Ile Thr Glu Met Ile Pro Glu Arg Asn Glu				
60 65 70				
CAA GGG CAA ACU CUA UGG AGU AAA AUG AGU GAU GCC GGA UCG GAU CGU	291			
Gln Gly Gln Thr Leu Trp Ser Lys Met Ser Asp Ala Gly Ser Asp Arg				
75 80 85				
GUG AUG GUA UCA CCU CUG GCU GUG ACA UGG UGG AAU AGA AAU GGA CCA	339			
Val Met Val Ser Pro Leu Ala Val Thr Trp Trp Asn Arg Asn Gly Pro				
90 95 100				



AUG Met 105	ACA Thr	AGU Ser	ACG Thr	GUU Val	CAU His 110	UAU Tyr	CCA Pro	AAA Lys	AUC Ile 115	UAC Tyr 115	AAA Lys	ACU Thr	UAU Tyr	UUU Phe	GAG Glu 120	387
AAA Lys	GUC Val	GAA Glu	AGG Arg	UUA Leu 125	AAA Lys	CAU His	GGA Gly	ACC Thr	UUU Phe 130	GGC Gly	CCU Pro	GUC Val	CAU His	UUU Phe 135	AGA Arg	435
AAC Asn	CAA Gln	GUC Val	AAA Lys 140	AUA Ile	CGC Arg	CGA Arg	AGA Arg	GUU Val 145	GAC Asp	AUA Ile	AAU Asn	CCU Pro	GGU Gly	CAU His	GCA Ala	483
GAC Asp	CUC Leu	AGU Ser 155	GCC Ala	AAG Lys	GAG Glu	GCA Ala	CAG Gln 160	GAU Asp	GUA Val	AUC Ile	AUG Met	GAA Glu 165	GUU Val	GUU Val	UUC Phe	531
CCU Pro 170	AAC Asn	GAA Glu	GUG Val	GGG Gly	GCC Ala	AGG Arg 175	AUA Ile	CUA Leu	ACG Thr	UCG Ser	GAA Glu 180	UCG Ser	CAA Gln	UUA Leu	ACA Thr	579
AUA Ile 185	ACC Thr	AAA Lys	GAG Glu	AAA Lys	AAA Lys 190	GAA Glu	GAA Glu	CUC Leu	CAG Gln	GAU Asp 195	UGC Cys	AAA Lys	AUU Ile	UCA Ser	CCU Pro 200	627
UUG Leu	AUG Met	GUU Val	GCG Ala	UAC Tyr 205	AUG Met	UUA Leu	GAG Glu	AGA Arg	GAA Glu 210	CUU Leu	GUC Val	CGA Arg	AAA Lys	ACG Thr 215	AGA Arg	675
UUU Phe	CUC Leu	CCA Pro	GUU Val 220	GCU Ala	GGU Gly	GGA Gly	ACA Thr	AGC Ser 225	AGU Ser	GUG Val	UAC Tyr	AUU Ile	GAA Glu 230	GUG Val	UUG Leu	723
CAC His	UUG Leu	ACU Thr 235	CAA Gln	GGA Gly	ACA Thr	UGC Cys	UGG Trp 240	GAA Glu	CAG Gln	AUG Met	UAC Tyr	ACU Thr 245	CCA Pro	GGU Gly	GGA Gly	771
GAA Glu 250	GUG Val	AGG Arg	AAU Asn	GAU Asp	GAU Asp	GUU Val 255	GAU Asp	CAA Gln	AGU Ser	CUA Leu	AUU Ile 260	AUU Ile	GCA Ala	GCC Ala	AGG Arg	819
AGC Ser 265	AUA Ile	GUG Val	AGA Arg	AGA Arg	GCA Ala 270	GCA Ala	GUA Val	UCA Ser	GCA Ala	GAU Asp 275	CCA Pro	CUA Leu	GCA Ala	UCU Ser	UUA Leu 280	867
UUG Leu	GAG Glu	AUG Met	UGC Cys	CAC His 285	AGC Ser	ACA Thr	CAG Gln	AUU Ile	GGC Gly 290	GGG Gly	ACA Thr	AGG Arg	AUG Met	GUG Val 295	GAC Asp	915
AUU Ile	CUU Leu	AGG Arg	CAG Gln 300	AAC Asn	CCA Pro	ACA Thr	GAA Glu	GAG Glu 305	CAA Gln	GCU Ala	GUG Val	GAA Glu	AUA Ile 310	UGC Cys	AAG Lys	963
GCU Ala	GCA Ala	AUG Met 315	GGA Gly	CUG Leu	AGG Arg	AUC Ile	AGC Ser 320	UCA Ser	UCC Ser	UUC Phe	AGU Ser	UUU Phe 325	GGC Gly	GGG Gly	UUC Phe	1011



ACA Thr	UUU Phe	AAG Lys	AGA Arg	ACA Thr	AGC Ser	GGA Gly	UCA Ser	UCA Ser	GUC Val	AAG Lys	AGA Arg	GAG Glu	GAA Glu	GAA Glu	GUG Val	1059
	330					335					340					
CUU Leu	ACG Thr	GGC Gly	AAU Asn	CUU Leu	CAA Gln	ACA Thr	UUG Leu	AAA Lys	AUA Ile	AGG Arg	GUG Val	CAU His	GAG Glu	GGA Gly	UAC Tyr	1107
	345				350					355					360	
GAG Glu	GAG Glu	UUC Phe	ACA Thr	AUG Met	GUU Val	GGG Gly	AAA Lys	AGG Arg	GCA Ala	ACA Thr	GCU Ala	AUA Ile	CUC Leu	AGA Arg	AAA Lys	1155
				365					370					375		
GCA Ala	ACC Thr	AGG Arg	AGA Arg	UUG Leu	AUU Ile	CAG Gln	CUG Leu	AUU Ile	GUG Val	AGU Ser	GGA Gly	AGA Arg	GAC Asp	GAA Glu	CAG Gln	1203
			380					385					390			
UCG Ser	AUA Ile	GCU Ala	GAA Glu	GCA Ala	AUA Ile	AUU Ile	GUG Val	GCC Ala	AUG Met	GUA Val	UUU Phe	UCA Ser	CAA Gln	GAA Glu	GAU Asp	1251
		395					400					405				
UGU Cys	AUG Met	AUA Ile	AAA Lys	GCA Ala	GUU Val	AGA Arg	GGU Gly	GAU Asp	CUG Leu	AAU Asn	UUC Phe	GUU Val	AAU Asn	AGG Arg	GCA Ala	1299
	410					415					420					
AAU Asn	CAG Gln	CGA Arg	UUG Leu	AAU Asn	CCC Pro	AUG Met	CAU His	CAA Gln	CUU Leu	UUA Leu	AGA Arg	CAU His	UUU Phe	CAG Gln	AAG Lys	1347
	425				430					435					440	
GAU Asp	GCG Ala	AAA Lys	GUG Val	CUU Leu	UUU Phe	CAA Gln	AAU Asn	UGG Trp	GGA Gly	AUU Ile	GAA Glu	CAU His	AUC Ile	GAC Asp	AAU Asn	1395
				445					450					455		
GUG Val	AUG Met	GGA Gly	AUG Met	AUU Ile	GGG Gly	GUA Val	UUA Leu	CCA Pro	GAC Asp	AUG Met	ACU Thr	CCA Pro	AGC Ser	ACA Thr	GAG Glu	1443
			460					465					470			
AUG Met	UCA Ser	AUG Met	AGA Arg	GGG Gly	GUA Val	AGA Arg	GUC Val	AGC Ser	AAA Lys	AUG Met	GGC Gly	GUA Val	GAU Asp	GAA Glu	UAC Tyr	1491
		475					480					485				
UCC Ser	AGC Ser	GCG Ala	GAG Glu	AGA Arg	GUA Val	GUG Val	GUG Val	AGC Ser	AUU Ile	GAC Asp	CGG Arg	UUU Phe	UUG Leu	AGA Arg	GUU Val	1539
	490					495					500					
CGA Arg	GAC Asp	CAA Gln	CGA Arg	GGA Gly	AAU Asn	GUA Val	CUA Leu	CUA Leu	UCU Ser	CCU Pro	GAG Glu	GAG Glu	GUC Val	AGU Ser	GAA Glu	1587
	505				510					515					520	
ACA Thr	CAG Gln	GGA Gly	ACA Thr	GAG Glu	AAA Lys	CUG Leu	ACA Thr	AUA Ile	ACU Thr	UAC Tyr	UCA Ser	UCG Ser	UCA Ser	AUG Met	AUG Met	1635
				525					530					535		
UGG Trp	GAG Glu	AUU Ile	AAU Asn	GGC Gly	CCU Pro	GAG Glu	UCA Ser	GUG Val	UUG Leu	GUC Val	AAU Asn	ACC Thr	UAU Tyr	CAG Gln	UGG Trp	1683
			540					545						550		



[illegible]



(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 759 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Met Glu Arg Ile Lys Glu Leu Arg Asn Leu Met Ser Gln Ser Arg Thr  
1 5 10 15  
Arg Glu Ile Leu Thr Lys Thr Thr Val Asp His Met Ala Ile Ile Lys  
20 25 30  
Lys Tyr Thr Ser Gly Arg Gln Glu Lys Asn Pro Ser Leu Arg Met Lys  
35 40 45  
Trp Met Met Ala Met Lys Tyr Pro Ile Thr Ala Asp Lys Arg Ile Thr  
50 55 60  
Glu Met Ile Pro Glu Arg Asn Glu Gln Gly Gln Thr Leu Trp Ser Lys  
65 70 75 80  
Met Ser Asp Ala Gly Ser Asp Arg Val Met Val Ser Pro Leu Ala Val  
85 90 95  
Thr Trp Trp Asn Arg Asn Gly Pro Met Thr Ser Thr Val His Tyr Pro  
100 105 110  
Lys Ile Tyr Lys Thr Tyr Phe Glu Lys Val Glu Arg Leu Lys His Gly  
115 120 125  
Thr Phe Gly Pro Val His Phe Arg Asn Gln Val Lys Ile Arg Arg Arg  
130 135 140  
Val Asp Ile Asn Pro Gly His Ala Asp Leu Ser Ala Lys Glu Ala Gln  
145 150 155 160  
Asp Val Ile Met Glu Val Val Phe Pro Asn Glu Val Gly Ala Arg Ile  
165 170 175  
Leu Thr Ser Glu Ser Gln Leu Thr Ile Thr Lys Glu Lys Lys Glu Glu  
180 185 190  
Leu Gln Asp Cys Lys Ile Ser Pro Leu Met Val Ala Tyr Met Leu Glu  
195 200 205



Arg	Glu	Leu	Val	Arg	Lys	Thr	Arg	Phe	Leu	Pro	Val	Ala	Gly	Gly	Thr
210						215					220				
Ser	Ser	Val	Tyr	Ile	Glu	Val	Leu	His	Leu	Thr	Gln	Gly	Thr	Cys	Trp
225					230					235					240
Glu	Gln	Met	Tyr	Thr	Pro	Gly	Gly	Glu	Val	Arg	Asn	Asp	Asp	Val	Asp
				245					250					255	
Gln	Ser	Leu	Ile	Ile	Ala	Ala	Arg	Ser	Ile	Val	Arg	Arg	Ala	Ala	Val
			260					265					270		
Ser	Ala	Asp	Pro	Leu	Ala	Ser	Leu	Leu	Glu	Met	Cys	His	Ser	Thr	Gln
		275					280					285			
Ile	Gly	Gly	Thr	Arg	Met	Val	Asp	Ile	Leu	Arg	Gln	Asn	Pro	Thr	Glu
	290					295					300				
Glu	Gln	Ala	Val	Glu	Ile	Cys	Lys	Ala	Ala	Met	Gly	Leu	Arg	Ile	Ser
305					310					315					320
Ser	Ser	Phe	Ser	Phe	Gly	Gly	Phe	Thr	Phe	Lys	Arg	Thr	Ser	Gly	Ser
				325					330					335	
Ser	Val	Lys	Arg	Glu	Glu	Glu	Val	Leu	Thr	Gly	Asn	Leu	Gln	Thr	Leu
			340					345					350		
Lys	Ile	Arg	Val	His	Glu	Gly	Tyr	Glu	Glu	Phe	Thr	Met	Val	Gly	Lys
		355					360					365			
Arg	Ala	Thr	Ala	Ile	Leu	Arg	Lys	Ala	Thr	Arg	Arg	Leu	Ile	Gln	Leu
	370					375					380				
Ile	Val	Ser	Gly	Arg	Asp	Glu	Gln	Ser	Ile	Ala	Glu	Ala	Ile	Ile	Val
385					390					395					400
Ala	Met	Val	Phe	Ser	Gln	Glu	Asp	Cys	Met	Ile	Lys	Ala	Val	Arg	Gly
				405					410					415	
Asp	Leu	Asn	Phe	Val	Asn	Arg	Ala	Asn	Gln	Arg	Leu	Asn	Pro	Met	His
			420					425					430		
Gln	Leu	Leu	Arg	His	Phe	Gln	Lys	Asp	Ala	Lys	Val	Leu	Phe	Gln	Asn
		435					440					445			
Trp	Gly	Ile	Glu	His	Ile	Asp	Asn	Val	Met	Gly	Met	Ile	Gly	Val	Leu
	450					455					460				
Pro	Asp	Met	Thr	Pro	Ser	Thr	Glu	Met	Ser	Met	Arg	Gly	Val	Arg	Val
465					470					475					480
Ser	Lys	Met	Gly	Val	Asp	Glu	Tyr	Ser	Ser	Ala	Glu	Arg	Val	Val	Val
				485					490					495	



Ser Ile Asp Arg Phe Leu Arg Val Arg Asp Gln Arg Gly Asn Val Leu  
500 505 510

Leu Ser Pro Glu Glu Val Ser Glu Thr Gln Gly Thr Glu Lys Leu Thr  
515 520 525

Ile Thr Tyr Ser Ser Ser Met Met Trp Glu Ile Asn Gly Pro Glu Ser  
530 535 540

Val Leu Val Asn Thr Tyr Gln Trp Ile Ile Arg Asn Trp Glu Thr Val  
545 550 555 560

Lys Ile Gln Trp Ser Gln Asn Pro Thr Met Leu Tyr Asn Lys Met Glu  
565 570 575

Phe Glu Pro Phe Gln Ser Leu Val Pro Lys Ala Ile Arg Gly Gln Tyr  
580 585 590

Ser Gly Phe Val Arg Thr Leu Phe Gln Gln Met Arg Asp Val Leu Gly  
595 600 605

Thr Phe Asp Thr Thr Gln Ile Ile Lys Leu Leu Pro Phe Ala Ala Ala  
610 615 620

Pro Pro Lys Gln Ser Arg Met Gln Phe Ser Ser Leu Thr Val Asn Val  
625 630 635 640

Arg Gly Ser Gly Met Arg Ile Leu Val Arg Gly Asn Ser Pro Ile Phe  
645 650 655

Asn Tyr Asn Lys Thr Thr Lys Arg Leu Thr Ile Leu Gly Lys Asp Ala  
660 665 670

Gly Thr Leu Thr Glu Asp Pro Asp Glu Gly Thr Ser Gly Val Glu Ser  
675 680 685

Ala Val Leu Arg Gly Phe Leu Ile Leu Gly Lys Glu Asp Arg Arg Tyr  
690 695 700

Gly Pro Ala Leu Ser Ile Asn Glu Leu Ser Asn Leu Ala Lys Gly Glu  
705 710 715 720

Lys Ala Asn Val Leu Ile Gly Gln Gly Asp Val Val Leu Val Met Lys  
725 730 735

Arg Lys Arg Asn Ser Ser Ile Leu Thr Asp Ser Gln Thr Ala Thr Lys  
740 745 750

Arg Ile Arg Met Ala Ile Asn  
755



(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2341 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: wild type A/Ann Arbor/6/60 (H2N2) Egg Passage 2(3)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: PB1

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(123, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2]).

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(486, "u")
- (D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3); c in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])



(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(1195, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: mutation

(B) LOCATION: replace(1276, "a")

(D) OTHER INFORMATION: /note= "g in ca "master" strain; a in  
wt2(3); g in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(1395, "u")

(D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3); g in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(1766, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])



(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(2005, "a")

(D) OTHER INFORMATION: /note= "a in ca "master" strain and in  
wt2(3); g in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(2019, "u")

(D) OTHER INFORMATION: /note= "u in ca "master" strain and in  
wt2(3); c in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 25..2295

(D) OTHER INFORMATION: /product= "polymerase basic 1"  
/gene= "PB1"  
/note= "polymerase basic 1"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G

(B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus

(C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA

(G) DATE: 1993

(K) RELEVANT RESIDUES IN SEQ ID NO:31: FROM 1 TO 2341



(x) PUBLICATION INFORMATION:

(A) AUTHORS: Cox, N J  
 Kitame, F  
 Kendal, A P  
 Maassab, H F  
 Naeve, C

(B) TITLE: Identification of sequence changes in the cold-adapted  
 live attenuated influenza vaccine strain

(C) JOURNAL: Virology

(D) VOLUME: 167

(F) PAGES: 554-567

(G) DATE: 1988

(K) RELEVANT RESIDUES IN SEQ ID NO:31: FROM 1 TO 2341

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AGCGAAAGCA GGCAAACCAU UUGA AUG GAU GUC AAU CCG ACC UUA CUU UUC	51
Met Asp Val Asn Pro Thr Leu Leu Phe	
1 5	
UUG AAA GUU CCA GCG CAA AAU GCC AUA AGU ACU ACA UUC CCU UAU ACU	99
Leu Lys Val Pro Ala Gln Asn Ala Ile Ser Thr Thr Phe Pro Tyr Thr	
10 15 20 25	
GGA GAU CCU CCA UAC AGC CAU GGG ACA GGA ACA GGA UAC ACC AUG GAC	147
Gly Asp Pro Pro Tyr Ser His Gly Thr Gly Thr Gly Tyr Thr Met Asp	
30 35 40	
ACA GUC AAC AGA ACA CAU CAA UAU UCA GAA AAG GGG AAG UGG ACA ACA	195
Thr Val Asn Arg Thr His Gln Tyr Ser Glu Lys Gly Lys Trp Thr Thr	
45 50 55	
AAC ACG GAA ACU GGA GCG CAC CAA CUU AAC CCA AUU GAU GGA CCA CUA	243
Asn Thr Glu Thr Gly Ala His Gln Leu Asn Pro Ile Asp Gly Pro Leu	
60 65 70	
CCU GAG GAC AAU GAA CCA AGU GGA UAU GCA CAA ACA GAC UGC GUC CUG	291
Pro Glu Asp Asn Glu Pro Ser Gly Tyr Ala Gln Thr Asp Cys Val Leu	
75 80 85	
GAA GCA AUG GCU UUC CUU GAA GAA UCC CAC CCA GGA AUC UUU GAA AAC	339
Glu Ala Met Ala Phe Leu Glu Glu Ser His Pro Gly Ile Phe Glu Asn	
90 95 100 105	



UCG	UGU	CUU	GAA	ACG	AUG	GAA	GUU	AUU	CAA	CAA	ACA	AGA	GUG	GAC	AAA	387
Ser	Cys	Leu	Glu	Thr	Met	Glu	Val	Ile	Gln	Gln	Thr	Arg	Val	Asp	Lys	
				110					115					120		
CUG	ACC	CAA	GGU	CGU	CAG	ACC	UAU	GAU	UGG	ACA	UUG	AAC	AGA	AAU	CAG	435
Leu	Thr	Gln	Gly	Arg	Gln	Thr	Tyr	Asp	Trp	Thr	Leu	Asn	Arg	Asn	Gln	
			125					130					135			
CCG	GCU	GCA	ACU	GCG	CUA	GCC	AAC	ACU	AUA	GAG	GUC	UUC	AGA	UCG	AAU	483
Pro	Ala	Ala	Thr	Ala	Leu	Ala	Asn	Thr	Ile	Glu	Val	Phe	Arg	Ser	Asn	
		140					145					150				
GGU	CUG	ACA	GCU	AAU	GAA	UCG	GGA	AGG	CUA	AUA	GAU	UUC	CUC	AAG	GAU	531
Gly	Leu	Thr	Ala	Asn	Glu	Ser	Gly	Arg	Leu	Ile	Asp	Phe	Leu	Lys	Asp	
	155					160					165					
GUG	AUA	GAA	UCA	AUG	GAU	AAA	GAG	GAG	AUG	GAA	AUC	ACA	ACA	CAC	UUC	579
Val	Ile	Glu	Ser	Met	Asp	Lys	Glu	Glu	Met	Glu	Ile	Thr	Thr	His	Phe	
170					175					180					185	
CAA	AGA	AAA	AGA	AGA	GUA	AGA	GAC	AAC	AUG	ACC	AAG	AAA	AUG	GUC	ACA	627
Gln	Arg	Lys	Arg	Arg	Val	Arg	Asp	Asn	Met	Thr	Lys	Lys	Met	Val	Thr	
				190					195					200		
CAA	CGA	ACA	AUA	GGA	AAG	AAG	AAG	CAA	AGA	UUG	AAC	AAG	AGA	AGC	UAU	675
Gln	Arg	Thr	Ile	Gly	Lys	Lys	Lys	Gln	Arg	Leu	Asn	Lys	Arg	Ser	Tyr	
			205					210					215			
CUA	AUA	AGA	GCA	CUG	ACA	UUG	AAC	ACA	AUG	ACU	AAA	GAU	GCA	GAG	AGA	723
Leu	Ile	Arg	Ala	Leu	Thr	Leu	Asn	Thr	Met	Thr	Lys	Asp	Ala	Glu	Arg	
		220					225					230				
GGU	AAA	UUA	AAG	AGA	AGA	GCA	AUU	GCA	ACA	CCC	GGU	AUG	CAG	AUC	AGA	771
Gly	Lys	Leu	Lys	Arg	Arg	Ala	Ile	Ala	Thr	Pro	Gly	Met	Gln	Ile	Arg	
	235					240					245					
GGG	UUC	GUG	UAC	UUU	GUC	GAA	ACA	CUA	GCG	AGA	AGU	AUU	UGU	GAG	AAG	819
Gly	Phe	Val	Tyr	Phe	Val	Glu	Thr	Leu	Ala	Arg	Ser	Ile	Cys	Glu	Lys	
250					255					260					265	
CUU	GAA	CAG	UCU	GGG	CUU	CCG	GUU	GGA	GGU	AAU	GAA	AAG	AAG	GCU	AAA	867
Leu	Glu	Gln	Ser	Gly	Leu	Pro	Val	Gly	Gly	Asn	Glu	Lys	Lys	Ala	Lys	
				270					275					280		
CUG	GCA	AAU	GUU	GUG	CGA	AAA	AUG	AUG	ACU	AAU	UCA	CAA	GAC	ACA	GAG	915
Leu	Ala	Asn	Val	Val	Arg	Lys	Met	Met	Thr	Asn	Ser	Gln	Asp	Thr	Glu	
			285					290					295			
CUC	UCU	UUC	ACA	AUU	ACU	GGA	GAC	AAU	ACC	AAA	UGG	AAU	GAG	AAU	CAA	963
Leu	Ser	Phe	Thr	Ile	Thr	Gly	Asp	Asn	Thr	Lys	Trp	Asn	Glu	Asn	Gln	
		300					305					310				
AAU	CCU	CGG	AUG	UUC	CUG	GCG	AUG	AUA	ACA	UAC	AUC	ACA	AGA	AAU	CAA	1011
Asn	Pro	Arg	Met	Phe	Leu	Ala	Met	Ile	Thr	Tyr	Ile	Thr	Arg	Asn	Gln	
	315					320					325					



CCU	GAA	UGG	UUU	AGA	AAC	GUC	CUG	AGC	AUC	GCA	CCU	AUA	AUG	UUC	UCA	1059
Pro	Glu	Trp	Phe	Arg	Asn	Val	Leu	Ser	Ile	Ala	Pro	Ile	Met	Phe	Ser	
330					335					340					345	
AAU	AAA	AUG	GCA	AGA	CUA	GGG	AAA	GGA	UAC	AUG	UUC	AAA	AGC	AAG	AGC	1107
Asn	Lys	Met	Ala	Arg	Leu	Gly	Lys	Gly	Tyr	Met	Phe	Lys	Ser	Lys	Ser	
				350					355					360		
AUG	AAG	CUC	CGA	ACA	CAA	AUA	CCA	GCA	GAA	AUG	CUA	GCA	AGU	AUU	GAC	1155
Met	Lys	Leu	Arg	Thr	Gln	Ile	Pro	Ala	Glu	Met	Leu	Ala	Ser	Ile	Asp	
			365					370					375			
CUG	AAA	UAC	UUU	AAU	GAA	UCA	ACA	AGA	AAG	AAA	AUC	GAG	GAA	AUA	AGG	1203
Leu	Lys	Tyr	Phe	Asn	Glu	Ser	Thr	Arg	Lys	Lys	Ile	Glu	Glu	Ile	Arg	
		380					385					390				
CCU	CUC	CUA	AUA	GAU	GGC	ACA	GUC	UCA	UUG	AGU	CCU	GGA	AUG	AUG	AUG	1251
Pro	Leu	Leu	Ile	Asp	Gly	Thr	Val	Ser	Leu	Ser	Pro	Gly	Met	Met	Met	
	395					400					405					
GGC	AUG	UUC	AAC	AUG	CUA	AGU	ACA	AUC	UUA	GGA	GUC	UCA	AUC	CUG	AAU	1299
Gly	Met	Phe	Asn	Met	Leu	Ser	Thr	Ile	Leu	Gly	Val	Ser	Ile	Leu	Asn	
410					415					420					425	
CUU	GGA	CAA	AAG	AAG	UAC	ACC	AAA	ACA	ACA	UAC	UGG	UGG	GAC	GGA	CUC	1347
Leu	Gly	Gln	Lys	Lys	Tyr	Thr	Lys	Thr	Thr	Tyr	Trp	Trp	Asp	Gly	Leu	
				430					435					440		
CAA	UCC	UCU	GAU	GAC	UUC	GCC	CUC	AUA	GUG	AAU	GCA	CCA	AAU	CAU	GAU	1395
Gln	Ser	Ser	Asp	Asp	Phe	Ala	Leu	Ile	Val	Asn	Ala	Pro	Asn	His	Asp	
			445					450					455			
GGA	AUA	CAA	GCA	GGG	GUG	GAU	AGA	UUC	UAC	AGA	ACC	UGC	AAG	CUA	GUC	1443
Gly	Ile	Gln	Ala	Gly	Val	Asp	Arg	Phe	Tyr	Arg	Thr	Cys	Lys	Leu	Val	
		460					465					470				
GGA	AUC	AAU	AUG	AGC	AAA	AAG	AAG	UCC	UAC	AUA	AAU	AGG	ACA	GGG	ACA	1491
Gly	Ile	Asn	Met	Ser	Lys	Lys	Lys	Ser	Tyr	Ile	Asn	Arg	Thr	Gly	Thr	
	475					480					485					
UUU	GAA	UUC	ACA	AGC	UUU	UUC	UAU	CGC	UAU	GGA	UUU	GUA	GCC	AAU	UUU	1539
Phe	Glu	Phe	Thr	Ser	Phe	Phe	Tyr	Arg	Tyr	Gly	Phe	Val	Ala	Asn	Phe	
490					495					500					505	
AGC	AUG	GAG	CUG	CCC	AGC	UUU	GGA	GUG	UCU	GGA	AUU	AAU	GAA	UCG	GCU	1587
Ser	Met	Glu	Leu	Pro	Ser	Phe	Gly	Val	Ser	Gly	Ile	Asn	Glu	Ser	Ala	
				510					515					520		
GAU	AUG	AGC	AUU	GGG	GUA	ACA	GUG	AUA	AAG	AAC	AAC	AUG	AUA	AAC	AAU	1635
Asp	Met	Ser	Ile	Gly	Val	Thr	Val	Ile	Lys	Asn	Asn	Met	Ile	Asn	Asn	
			525					530					535			
GAC	CUU	GGG	CCA	GCA	ACA	GCC	CAA	CUG	GCU	CUU	CAA	CUA	UUC	AUC	AAA	1683
Asp	Leu	Gly	Pro	Ala	Thr	Ala	Gln	Leu	Ala	Leu	Gln	Leu	Phe	Ile	Lys	
		540					545					550				



AGCUUGUCCU UCAUGAAAAA AUGCCUUGUU UCUACU 2341



(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 757 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met Asp Val Asn Pro Thr Leu Leu Phe Leu Lys Val Pro Ala Gln Asn  
1 5 10 15  
Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Asp Pro Pro Tyr Ser His  
20 25 30  
Gly Thr Gly Thr Gly Tyr Thr Met Asp Thr Val Asn Arg Thr His Gln  
35 40 45  
Tyr Ser Glu Lys Gly Lys Trp Thr Thr Asn Thr Glu Thr Gly Ala His  
50 55 60  
Gln Leu Asn Pro Ile Asp Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser  
65 70 75 80  
Gly Tyr Ala Gln Thr Asp Cys Val Leu Glu Ala Met Ala Phe Leu Glu  
85 90 95  
Glu Ser His Pro Gly Ile Phe Glu Asn Ser Cys Leu Glu Thr Met Glu  
100 105 110  
Val Ile Gln Gln Thr Arg Val Asp Lys Leu Thr Gln Gly Arg Gln Thr  
115 120 125  
Tyr Asp Trp Thr Leu Asn Arg Asn Gln Pro Ala Ala Thr Ala Leu Ala  
130 135 140  
Asn Thr Ile Glu Val Phe Arg Ser Asn Gly Leu Thr Ala Asn Glu Ser  
145 150 155 160  
Gly Arg Leu Ile Asp Phe Leu Lys Asp Val Ile Glu Ser Met Asp Lys  
165 170 175  
Glu Glu Met Glu Ile Thr Thr His Phe Gln Arg Lys Arg Arg Val Arg  
180 185 190  
Asp Asn Met Thr Lys Lys Met Val Thr Gln Arg Thr Ile Gly Lys Lys  
195 200 205



Lys Gln Arg Leu Asn Lys Arg Ser Tyr Leu Ile Arg Ala Leu Thr Leu  
 210 215 220  
 Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala  
 225 230 235 240  
 Ile Ala Thr Pro Gly Met Gln Ile Arg Gly Phe Val Tyr Phe Val Glu  
 245 250 255  
 Thr Leu Ala Arg Ser Ile Cys Glu Lys Leu Glu Gln Ser Gly Leu Pro  
 260 265 270  
 Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ala Asn Val Val Arg Lys  
 275 280 285  
 Met Met Thr Asn Ser Gln Asp Thr Glu Leu Ser Phe Thr Ile Thr Gly  
 290 295 300  
 Asp Asn Thr Lys Trp Asn Glu Asn Gln Asn Pro Arg Met Phe Leu Ala  
 305 310 315 320  
 Met Ile Thr Tyr Ile Thr Arg Asn Gln Pro Glu Trp Phe Arg Asn Val  
 325 330 335  
 Leu Ser Ile Ala Pro Ile Met Phe Ser Asn Lys Met Ala Arg Leu Gly  
 340 345 350  
 Lys Gly Tyr Met Phe Lys Ser Lys Ser Met Lys Leu Arg Thr Gln Ile  
 355 360 365  
 Pro Ala Glu Met Leu Ala Ser Ile Asp Leu Lys Tyr Phe Asn Glu Ser  
 370 375 380  
 Thr Arg Lys Lys Ile Glu Glu Ile Arg Pro Leu Leu Ile Asp Gly Thr  
 385 390 395 400  
 Val Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu Ser  
 405 410 415  
 Thr Ile Leu Gly Val Ser Ile Leu Asn Leu Gly Gln Lys Lys Tyr Thr  
 420 425 430  
 Lys Thr Thr Tyr Trp Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala  
 435 440 445  
 Leu Ile Val Asn Ala Pro Asn His Asp Gly Ile Gln Ala Gly Val Asp  
 450 455 460  
 Arg Phe Tyr Arg Thr Cys Lys Leu Val Gly Ile Asn Met Ser Lys Lys  
 465 470 475 480  
 Lys Ser Tyr Ile Asn Arg Thr Gly Thr Phe Glu Phe Thr Ser Phe Phe  
 485 490 495



Tyr Arg Tyr Gly Phe Val Ala Asn Phe Ser Met Glu Leu Pro Ser Phe  
 500 505 510  
 Gly Val Ser Gly Ile Asn Glu Ser Ala Asp Met Ser Ile Gly Val Thr  
 515 520 525  
 Val Ile Lys Asn Asn Met Ile Asn Asn Asp Leu Gly Pro Ala Thr Ala  
 530 535 540  
 Gln Leu Ala Leu Gln Leu Phe Ile Lys Asp Tyr Arg Tyr Thr Tyr Arg  
 545 550 555 560  
 Cys His Arg Gly Asp Thr Gln Ile Gln Thr Arg Arg Ser Phe Glu Leu  
 565 570 575  
 Lys Lys Leu Trp Gly Gln Thr Arg Ser Lys Ala Gly Leu Leu Val Ser  
 580 585 590  
 Asp Gly Gly Pro Asn Leu Tyr Asn Ile Arg Asn Leu His Ile Pro Glu  
 595 600 605  
 Val Cys Leu Lys Trp Glu Leu Met Asp Glu Asp Tyr Gln Gly Arg Leu  
 610 615 620  
 Cys Asn Pro Leu Asn Pro Phe Val Ser His Lys Glu Ile Glu Ser Val  
 625 630 635 640  
 Asn Asn Ala Val Val Met Pro Ala His Gly Pro Ala Lys Ser Met Glu  
 645 650 655  
 Tyr Asp Ala Val Thr Thr Thr His Ser Trp Ile Pro Lys Arg Asn Arg  
 660 665 670  
 Ser Ile Leu Asn Thr Ser Gln Arg Gly Ile Leu Glu Asp Glu Gln Met  
 675 680 685  
 Tyr Gln Lys Cys Cys Asn Leu Phe Glu Lys Phe Phe Pro Ser Ser Ser  
 690 695 700  
 Tyr Arg Arg Pro Val Gly Ile Ser Ser Met Val Glu Ala Met Val Ser  
 705 710 715 720  
 Arg Ala Arg Ile Asp Ala Arg Ile Asp Phe Glu Ser Gly Arg Ile Lys  
 725 730 735  
 Lys Glu Glu Phe Ala Glu Ile Met Lys Ile Cys Ser Thr Ile Glu Glu  
 740 745 750  
 Leu Arg Arg Gln Lys  
 755



(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2233 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: wild type A/Ann Arbor/6/60 (H2N2) Egg Passage 2(3)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: PA

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(20, "c")
- (D) OTHER INFORMATION: /note= "c in ca "master" strain and in  
wt2(3); u in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

- (A) NAME/KEY: conflict
- (B) LOCATION: replace(75, "g")
- (D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); u in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])



(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(1861, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(2167..2168, "cc")

(D) OTHER INFORMATION: /note= "cc in ca "master" strain and in  
wt2(3); uu in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 25..2172

(D) OTHER INFORMATION: /product= "polymerase acidic protein"  
/gene= "PA"  
/note= "polymerase acidic protein"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G

(B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus

(C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA

(G) DATE: 1993

(K) RELEVANT RESIDUES IN SEQ ID NO:33: FROM 1 TO 2233



(x) PUBLICATION INFORMATION:

(A) AUTHORS: Cox, N J  
 Kitame, F  
 Kendal, A P  
 Maassab, H F  
 Naeve, C

(B) TITLE: Identification of sequence changes in the cold-adapted  
 live attenuated influenza strain, A/Ann  
 Arbor/6/60(H2N2)

(C) JOURNAL: Virology

(D) VOLUME: 167

(F) PAGES: 554-567

(G) DATE: 1988

(K) RELEVANT RESIDUES IN SEQ ID NO:33: FROM 1 TO 2233

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AGCGAAAGCA GGUACUGAUC CGAA AUG GAA GAU UUU GUG CGA CAA UGC UUC	51
Met Glu Asp Phe Val Arg Gln Cys Phe	
1 5	
AAU CCG AUG AUU GUC GAG CUU GCG GAA AAA GCA AUG AAA GAG UAU GGA	99
Asn Pro Met Ile Val Glu Leu Ala Glu Lys Ala Met Lys Glu Tyr Gly	
10 15 20 25	
GAG GAU CUG AAA AUC GAA ACA AAC AAA UUU GCA GCA AUA UGC ACU CAC	147
Glu Asp Leu Lys Ile Glu Thr Asn Lys Phe Ala Ala Ile Cys Thr His	
30 35 40	
UUG GAA GUA UGC UUC AUG UAU UCA GAU UUU CAU UUC AUC AAU GAG CAA	195
Leu Glu Val Cys Phe Met Tyr Ser Asp Phe His Phe Ile Asn Glu Gln	
45 50 55	
GGC GAG UCA AUA AUA GUA GAG CUU GAU GAU CCA AAU GCA CUU UUG AAG	243
Gly Glu Ser Ile Ile Val Glu Leu Asp Asp Pro Asn Ala Leu Leu Lys	
60 65 70	
CAC AGA UUU GAA AUA AUA GAG GGA AGA GAU CGC ACA AUG GCC UGG ACA	291
His Arg Phe Glu Ile Ile Glu Gly Arg Asp Arg Thr Met Ala Trp Thr	
75 80 85	
GUA GUA AAC AGU AUU UGC AAC ACU ACA GGA GCU GAG AAA CCG AAG UUU	339
Val Val Asn Ser Ile Cys Asn Thr Thr Gly Ala Glu Lys Pro Lys Phe	
90 95 100 105	



CUG	CCA	GAU	UUG	UAU	GAU	UAC	AAG	GAG	AAU	AGA	UUC	AUC	GAG	AUU	GGA	387
Leu	Pro	Asp	Leu	Tyr	Asp	Tyr	Lys	Glu	Asn	Arg	Phe	Ile	Glu	Ile	Gly	
			110						115					120		
GUG	ACA	AGG	AGG	GAA	GUC	CAC	AUA	UAC	UAU	CUU	GAA	AAG	GCC	AAU	AAA	435
Val	Thr	Arg	Arg	Glu	Val	His	Ile	Tyr	Tyr	Leu	Glu	Lys	Ala	Asn	Lys	
			125					130					135			
AUU	AAA	UCU	GAG	AAG	ACA	CAC	AUC	CAC	AUU	UUC	UCA	UUC	ACU	GGG	GAA	483
Ile	Lys	Ser	Glu	Lys	Thr	His	Ile	His	Ile	Phe	Ser	Phe	Thr	Gly	Glu	
		140					145					150				
GAA	AUG	GCC	ACA	AAG	GCC	GAC	UAC	ACU	CUC	GAU	GAG	GAA	AGC	AGG	GCU	531
Glu	Met	Ala	Thr	Lys	Ala	Asp	Tyr	Thr	Leu	Asp	Glu	Glu	Ser	Arg	Ala	
	155					160					165					
AGG	AUC	AAA	ACC	AGA	CUA	UUC	ACC	AUA	AGA	CAA	GAA	AUG	GCU	AGC	AGA	579
Arg	Ile	Lys	Thr	Arg	Leu	Phe	Thr	Ile	Arg	Gln	Glu	Met	Ala	Ser	Arg	
170					175					180					185	
GGC	CUC	UGG	GAU	UCC	UUU	CAU	CAG	UCC	GAA	AGA	GGC	GAA	GAA	ACA	AUU	627
Gly	Leu	Trp	Asp	Ser	Phe	His	Gln	Ser	Glu	Arg	Gly	Glu	Glu	Thr	Ile	
				190					195					200		
GAA	GAA	AGA	UUU	GAA	AUC	ACA	GGG	ACA	AUG	CGC	AGG	CUC	GCC	GAC	CAA	675
Glu	Glu	Arg	Phe	Glu	Ile	Thr	Gly	Thr	Met	Arg	Arg	Leu	Ala	Asp	Gln	
			205					210					215			
AGU	CUC	CCG	CCG	AAC	UUC	UCC	UGC	CUU	GAG	AAU	UUU	AGA	GCC	UAU	GUG	723
Ser	Leu	Pro	Pro	Asn	Phe	Ser	Cys	Leu	Glu	Asn	Phe	Arg	Ala	Tyr	Val	
		220					225					230				
GAU	GGA	UUC	GAA	CCG	AAC	GGC	UAC	AUU	GAG	GGC	AAG	CUU	UCU	CAA	AUG	771
Asp	Gly	Phe	Glu	Pro	Asn	Gly	Tyr	Ile	Glu	Gly	Lys	Leu	Ser	Gln	Met	
	235					240					245					
UCC	AAA	GAA	GUA	AAU	GCU	AAA	AUU	GAA	CCU	UUU	CUG	AAA	ACA	ACA	CCA	819
Ser	Lys	Glu	Val	Asn	Ala	Lys	Ile	Glu	Pro	Phe	Leu	Lys	Thr	Thr	Pro	
250				255						260					265	
AGA	CCA	AUU	AGA	CUU	CCG	GAU	GGG	CCU	CCU	UGU	UCU	CAG	CGG	UCC	AAA	867
Arg	Pro	Ile	Arg	Leu	Pro	Asp	Gly	Pro	Pro	Cys	Ser	Gln	Arg	Ser	Lys	
				270					275					280		
UUC	CUG	CUG	AUG	GAU	GCU	UUA	AAA	UUA	AGC	AUU	GAG	GAC	CCA	AGU	CAC	915
Phe	Leu	Leu	Met	Asp	Ala	Leu	Lys	Leu	Ser	Ile	Glu	Asp	Pro	Ser	His	
			285					290					295			
GAA	GGA	GAG	GGA	AUA	CCA	CUA	UAU	GAU	GCG	AUC	AAG	UGU	AUG	AGA	ACA	963
Glu	Gly	Glu	Gly	Ile	Pro	Leu	Tyr	Asp	Ala	Ile	Lys	Cys	Met	Arg	Thr	
		300					305					310				
UUC	UUU	GGA	UGG	AAA	GAA	CCC	UAU	GUU	GUU	AAA	CCA	CAC	GAA	AAG	GGA	1011
Phe	Phe	Gly	Trp	Lys	Glu	Pro	Tyr	Val	Val	Lys	Pro	His	Glu	Lys	Gly	
	315					320					325					



AUA Ile 330	AAU Asn	CCA Pro	AAU Asn	UAU Tyr	CUG Leu 335	CUG Leu	UCA Ser	UGG Trp	AAG Lys	CAA Gln 340	GUA Val	CUG Leu	GCA Ala	GAA Glu 345	CUG Leu 345	1059
CAG Gln	GAC Asp	AUU Ile	GAG Glu	AAU Asn 350	GAG Glu	GAG Glu	AAG Lys	AUU Ile	CCA Pro 355	AGA Arg	ACC Thr	AAA Lys	AAC Asn	AUG Met 360	AAG Lys	1107
AAA Lys	ACG Thr	AGU Ser	CAG Gln 365	CUA Leu	AAG Lys	UGG Trp	GCA Ala	CUU Leu 370	GGU Gly	GAG Glu	AAC Asn	AUG Met	GCA Ala 375	CCA Pro	GAG Glu	1155
AAG Lys	GUA Val	GAC Asp 380	UUU Phe	GAC Asp	GAC Asp	UGU Cys	AGA Arg 385	GAU Asp	GUA Val	AGC Ser	GAU Asp	UUG Leu 390	AAG Lys	CAA Gln	UAU Tyr	1203
GAU Asp 395	AGU Ser	GAU Asp	GAA Glu	CCU Pro	GAA Glu	UUA Leu 400	AGG Arg	UCA Ser	CUU Leu	UCA Ser	AGC Ser 405	UGG Trp	AUC Ile	CAG Gln	AAU Asn	1251
GAG Glu 410	UUC Phe	AAC Asn	AAG Lys	GCA Ala	UGC Cys 415	GAG Glu	CUG Leu	ACC Thr	GAU Asp 420	UCA Ser	AUC Ile	UGG Trp	AUA Ile	GAG Glu	CUC Leu 425	1299
GAU Asp	GAG Glu	AUU Ile	GGA Gly	GAA Glu 430	GAU Asp	GUG Val	GCU Ala	CCA Pro	AUU Ile 435	GAA Glu	CAC His	AUU Ile	GCA Ala	AGC Ser 440	AUG Met	1347
AGA Arg	AGG Arg	AAU Asn	UAC Tyr 445	UUC Phe	ACA Thr	GCA Ala	GAG Glu	GUG Val 450	UCU Ser	CAU His	UGC Cys	AGA Arg	GCC Ala 455	ACA Thr	GAA Glu	1395
UAU Tyr	AUA Ile	AUG Met 460	AAG Lys	GGG Gly	GUA Val	UAC Tyr	AUU Ile 465	AAU Asn	ACU Thr	GCC Ala	UUG Leu 470	CUU Leu	AAU Asn	GCA Ala	UCC Ser	1443
UGU Cys 475	GCA Ala	GCA Ala	AUG Met	GAC Asp	GAU Asp	UUC Phe 480	CAA Gln	CUA Leu	AUU Ile	CCC Pro	AUG Met 485	AUA Ile	AGC Ser	AAA Lys	UGU Cys	1491
AGA Arg 490	ACU Thr	AAA Lys	GAG Glu	GGA Gly	AGG Arg 495	CGA Arg	AAG Lys	ACC Thr	AAU Asn	UUA Leu 500	UAU Tyr	GGU Gly	UUC Phe	AUC Ile	AUA Ile 505	1539
AAA Lys	GGA Gly	AGA Arg	UCU Ser	CAC His 510	UUA Leu	AGG Arg	AAU Asn	GAC Asp	ACC Thr 515	GAC Asp	GUG Val	GUA Val	AAC Asn	UUU Phe 520	GUG Val	1587
AGC Ser	AUG Met	GAG Glu	UUU Phe 525	UCU Ser	CUC Leu	ACU Thr	GAC Asp	CCA Pro 530	AGA Arg	CUU Leu	GAG Glu	CCA Pro	CAC His 535	AAA Lys	UGG Trp	1635
GAG Glu	AAG Lys	UAC Tyr 540	UGU Cys	GUU Val	CUU Leu	GAG Glu	AUA Ile 545	GGA Gly	GAU Asp	AUG Met	CUA Leu	CUA Leu	AGA Arg	AGU Ser	GCC Ala	1683



[illegible]



(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 716 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Met	Glu	Asp	Phe	Val	Arg	Gln	Cys	Phe	Asn	Pro	Met	Ile	Val	Glu	Leu
1				5					10					15	
Ala	Glu	Lys	Ala	Met	Lys	Glu	Tyr	Gly	Glu	Asp	Leu	Lys	Ile	Glu	Thr
			20					25					30		
Asn	Lys	Phe	Ala	Ala	Ile	Cys	Thr	His	Leu	Glu	Val	Cys	Phe	Met	Tyr
		35					40					45			
Ser	Asp	Phe	His	Phe	Ile	Asn	Glu	Gln	Gly	Glu	Ser	Ile	Ile	Val	Glu
	50					55					60				
Leu	Asp	Asp	Pro	Asn	Ala	Leu	Leu	Lys	His	Arg	Phe	Glu	Ile	Ile	Glu
65					70					75					80
Gly	Arg	Asp	Arg	Thr	Met	Ala	Trp	Thr	Val	Val	Asn	Ser	Ile	Cys	Asn
				85					90					95	
Thr	Thr	Gly	Ala	Glu	Lys	Pro	Lys	Phe	Leu	Pro	Asp	Leu	Tyr	Asp	Tyr
			100					105					110		
Lys	Glu	Asn	Arg	Phe	Ile	Glu	Ile	Gly	Val	Thr	Arg	Arg	Glu	Val	His
		115					120					125			
Ile	Tyr	Tyr	Leu	Glu	Lys	Ala	Asn	Lys	Ile	Lys	Ser	Glu	Lys	Thr	His
	130					135					140				
Ile	His	Ile	Phe	Ser	Phe	Thr	Gly	Glu	Glu	Met	Ala	Thr	Lys	Ala	Asp
145					150					155					160
Tyr	Thr	Leu	Asp	Glu	Glu	Ser	Arg	Ala	Arg	Ile	Lys	Thr	Arg	Leu	Phe
			165						170					175	
Thr	Ile	Arg	Gln	Glu	Met	Ala	Ser	Arg	Gly	Leu	Trp	Asp	Ser	Phe	His
			180					185					190		
Gln	Ser	Glu	Arg	Gly	Glu	Glu	Thr	Ile	Glu	Glu	Arg	Phe	Glu	Ile	Thr
		195					200					205			



Gly	Thr	Met	Arg	Arg	Leu	Ala	Asp	Gln	Ser	Leu	Pro	Pro	Asn	Phe	Ser
	210					215					220				
Cys	Leu	Glu	Asn	Phe	Arg	Ala	Tyr	Val	Asp	Gly	Phe	Glu	Pro	Asn	Gly
225					230					235					240
Tyr	Ile	Glu	Gly	Lys	Leu	Ser	Gln	Met	Ser	Lys	Glu	Val	Asn	Ala	Lys
				245					250					255	
Ile	Glu	Pro	Phe	Leu	Lys	Thr	Thr	Pro	Arg	Pro	Ile	Arg	Leu	Pro	Asp
			260					265					270		
Gly	Pro	Pro	Cys	Ser	Gln	Arg	Ser	Lys	Phe	Leu	Leu	Met	Asp	Ala	Leu
		275					280					285			
Lys	Leu	Ser	Ile	Glu	Asp	Pro	Ser	His	Glu	Gly	Glu	Gly	Ile	Pro	Leu
	290					295					300				
Tyr	Asp	Ala	Ile	Lys	Cys	Met	Arg	Thr	Phe	Phe	Gly	Trp	Lys	Glu	Pro
305					310					315					320
Tyr	Val	Val	Lys	Pro	His	Glu	Lys	Gly	Ile	Asn	Pro	Asn	Tyr	Leu	Leu
				325					330					335	
Ser	Trp	Lys	Gln	Val	Leu	Ala	Glu	Leu	Gln	Asp	Ile	Glu	Asn	Glu	Glu
			340					345					350		
Lys	Ile	Pro	Arg	Thr	Lys	Asn	Met	Lys	Lys	Thr	Ser	Gln	Leu	Lys	Trp
		355					360					365			
Ala	Leu	Gly	Glu	Asn	Met	Ala	Pro	Glu	Lys	Val	Asp	Phe	Asp	Asp	Cys
	370					375					380				
Arg	Asp	Val	Ser	Asp	Leu	Lys	Gln	Tyr	Asp	Ser	Asp	Glu	Pro	Glu	Leu
385					390					395					400
Arg	Ser	Leu	Ser	Ser	Trp	Ile	Gln	Asn	Glu	Phe	Asn	Lys	Ala	Cys	Glu
				405					410					415	
Leu	Thr	Asp	Ser	Ile	Trp	Ile	Glu	Leu	Asp	Glu	Ile	Gly	Glu	Asp	Val
			420					425					430		
Ala	Pro	Ile	Glu	His	Ile	Ala	Ser	Met	Arg	Arg	Asn	Tyr	Phe	Thr	Ala
		435					440					445			
Glu	Val	Ser	His	Cys	Arg	Ala	Thr	Glu	Tyr	Ile	Met	Lys	Gly	Val	Tyr
	450					455					460				
Ile	Asn	Thr	Ala	Leu	Leu	Asn	Ala	Ser	Cys	Ala	Ala	Met	Asp	Asp	Phe
465					470					475					480
Gln	Leu	Ile	Pro	Met	Ile	Ser	Lys	Cys	Arg	Thr	Lys	Glu	Gly	Arg	Arg
				485					490					495	



Lys Thr Asn Leu Tyr Gly Phe Ile Ile Lys Gly Arg Ser His Leu Arg  
 500 505 510  
 Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr  
 515 520 525  
 Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu  
 530 535 540  
 Ile Gly Asp Met Leu Leu Arg Ser Ala Ile Gly Gln Val Ser Arg Pro  
 545 550 555 560  
 Met Phe Leu Tyr Val Arg Thr Asn Gly Thr Ser Lys Ile Lys Met Lys  
 565 570 575  
 Trp Gly Met Glu Met Arg Arg Cys Leu Leu Gln Ser Leu Gln Gln Ile  
 580 585 590  
 Glu Ser Met Ile Glu Ala Glu Ser Ser Val Lys Glu Lys Asp Met Thr  
 595 600 605  
 Lys Glu Phe Phe Glu Asn Lys Ser Glu Thr Trp Pro Ile Gly Glu Ser  
 610 615 620  
 Pro Lys Gly Val Glu Glu Gly Ser Ile Gly Lys Val Cys Arg Thr Leu  
 625 630 635 640  
 Leu Ala Lys Ser Val Phe Asn Ser Leu Tyr Ala Ser Pro Gln Leu Glu  
 645 650 655  
 Gly Phe Ser Ala Glu Ser Arg Lys Leu Leu Leu Val Val Gln Ala Leu  
 660 665 670  
 Arg Asp Asn Leu Glu Pro Gly Thr Phe Asp Leu Gly Gly Leu Tyr Glu  
 675 680 685  
 Ala Ile Glu Glu Cys Leu Ile Asn Asp Pro Trp Val Leu Leu Asn Ala  
 690 695 700  
 Ser Trp Phe Asn Ser Phe Leu Thr His Ala Pro Arg  
 705 710 715

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1773 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear



(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Influenza virus

(B) STRAIN: wild type A/Ann Arbor/6/60 (H2N2) Egg Passage 2(3)

(vii) IMMEDIATE SOURCE:

(B) CLONE: HA

(ix) FEATURE:

(A) NAME/KEY: mutation

(B) LOCATION: replace(144, "a")

(D) OTHER INFORMATION: /gene= "HA"  
/note= "u in ca "master" strain; a in  
wt2(3)"  
/citation= ([1])

(ix) FEATURE:

(A) NAME/KEY: mutation

(B) LOCATION: replace(455, "g")

(D) OTHER INFORMATION: /gene= "HA"  
/note= "a in ca "master" strain; g in  
wt2(3)"  
/citation= ([1])

(ix) FEATURE:

(A) NAME/KEY: mutation

(B) LOCATION: replace(729, "a")

(D) OTHER INFORMATION: /gene= "HA"  
/note= "c in ca "master" strain; a in  
wt2(3)"  
/citation= ([1])



(A) NAME/KEY: CDS

(B) LOCATION: 44..1729

(D) OTHER INFORMATION: /product= "hemagglutinin"  
/gene= "HA"  
/note= "hemagglutinin protein"  
/citation= ([1])

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G

(B) TITLE: Molecular and biological changes in the cold adapted master strain A/AA/6/60 (H2N2) influenza virus

(C) JOURNAL: Proceedings of the National Academy of Sciences of the USA

(G) DATE: 1993

(K) RELEVANT RESIDUES IN SEQ ID NO:35: FROM 1 TO 1773

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AGCAAAAGCA	GGGGUUAUAC	CAUAGACAAC	CAAAAGCAAA	ACA	AUG Met	GCC Ala	AUC Ile	AUU Ile		55						
UAU Tyr	CUC Leu	AUU Ile	CUC Leu	CUG Leu	UUC Phe	ACA Thr	GCA Ala	GUG Val	AGA Arg	GGG Gly	GAC Asp	AAG Lys	AUA Ile	UGC Cys	AUU Ile	103
	5				10					15					20	
GGA Gly	UAC Tyr	CAU His	GCC Ala	AAU Asn	AAU Asn	UCC Ser	ACA Thr	GAG Glu	ACG Thr	GUC Val	GAC Asp	ACA Thr	AAU Asn	CUA Leu	GAG Glu	151
				25					30					35		
CGG Arg	AAC Asn	GUC Val	ACU Thr	GUG Val	ACU Thr	CAU His	GCC Ala	AAG Lys	GAC Asp	AUU Ile	CUU Leu	GAG Glu	AAG Lys	ACC Thr	CAU His	199
			40					45					50			
AAC Asn	GGA Gly	AAG Lys	UUA Leu	UGC Cys	AAA Lys	CUA Leu	AAC Asn	GGA Gly	AUC Ile	CCU Pro	CCA Pro	CUU Leu	GAA Glu	CUA Leu	GGG Gly	247
		55					60					65				
GAC Asp	UGU Cys	AGC Ser	AUU Ile	GCC Ala	GGA Gly	UGG Trp	CUC Leu	CUU Leu	GGA Gly	AAU Asn	CCA Pro	GAA Glu	UGU Cys	GAU Asp	AGG Arg	295
	70					75					80					



CUU Leu 85	CUA Leu	AGU Ser	GUG Val	CCA Pro	GAA Glu 90	UGG Trp	UCC Ser	UAU Tyr	AUA Ile	AUG Met 95	GAG Glu	AAA Lys	GAA Glu	AAC Asn	CCG Pro 100	343
AGA Arg	AAC Asn	GGU Gly	UUG Leu	UGU Cys 105	UAU Tyr	CCA Pro	GGC Gly	AAC Asn	UUC Phe 110	AAU Asn	GAU Asp	UAU Tyr	GAA Glu	GAA Glu 115	UUG Leu	391
AAA Lys	CAU His	CUC Leu	CUC Leu 120	AGC Ser	AGC Ser	GUG Val	AAA Lys	CAU His 125	UUC Phe	GAG Glu	AAA Lys	GUA Val	AAG Lys 130	AUU Ile	CUG Leu	439
CCC Pro	AAA Lys	GAU Asp 135	AGA Arg	UGG Trp	GCA Ala	CAG Gln	CAU His 140	ACA Thr	ACA Thr	ACU Thr	GGA Gly	GGU Gly 145	UCA Ser	CAG Gln	GCC Ala	487
UGC Cys 150	GCG Ala	GUG Val	UCU Ser	GGU Gly	AAU Asn	CCA Pro 155	UCA Ser	UUC Phe	UUC Phe	AGG Arg	AAC Asn 160	AUG Met	GUC Val	UGG Trp	CUG Leu	535
ACA Thr 165	GAG Glu	GAA Glu	GGA Gly	UCA Ser	AAU Asn 170	UAU Tyr	CCG Pro	GUU Val	GCC Ala	AAA Lys 175	GGA Gly	UCG Ser	UAC Tyr	AAC Asn	AAU Asn 180	583
ACA Thr	AGC Ser	GGA Gly	GAA Glu	CAA Gln 185	AUG Met	CUA Leu	AUA Ile	AUU Ile	UGG Trp 190	GGG Gly	GUG Val	CAC His	CAU His	CCC Pro 195	AUU Ile	631
GAU Asp	GAG Glu	ACA Thr	GAA Glu 200	CAA Gln	AGA Arg	ACA Thr	UUG Leu 205	UAC Tyr	CAG Gln	AAU Asn	GUG Val	GGA Gly	ACC Thr 210	UAU Tyr	GUU Val	679
UCC Ser	GUA Val	GGC Gly 215	ACA Thr	UCA Ser	ACA Thr	UUG Leu	AAC Asn 220	AAA Lys	AGG Arg	UCA Ser	ACC Thr	CCA Pro 225	GAA Glu	AUA Ile	GCA Ala	727
AAA Lys 230	AGG Arg	CCU Pro	AAA Lys	GUG Val	AAU Asn	GGA Gly 235	CUA Leu	GGA Gly	AGU Ser	AGA Arg	AUG Met 240	GAA Glu	UUC Phe	UCU Ser	UGG Trp	775
ACC Thr 245	CUC Leu	UUG Leu	GAU Asp	AUG Met	UGG Trp 250	GAC Asp	ACC Thr	AUA Ile	AAU Asn	UUU Phe 255	GAG Glu	AGU Ser	ACU Thr	GGU Gly	AAU Asn 260	823
CUA Leu	AUU Ile	GCA Ala	CCA Pro	GAG Glu 265	UAU Tyr	GGA Gly	UUC Phe	AAA Lys	AUA Ile 270	UCG Ser	AAA Lys	AGA Arg	GGU Gly	AGU Ser 275	UCU Ser	871
GGG Gly	AUC Ile	AUG Met	AAA Lys 280	ACA Thr	GAA Glu	GGA Gly	ACA Thr	CUU Leu 285	GAG Glu	AAC Asn	UGU Cys	GAG Glu	ACC Thr	AAA Lys	UGC Cys	919
CAA Gln	ACU Thr	CCU Pro 295	UUG Leu	GGA Gly	GCA Ala	AUA Ile	AAU Asn 300	ACA Thr	ACA Thr	UUG Leu	CCU Pro	UUU Phe 305	CAC His	AAU Asn	GUC Val	967



CAC His	CCA Pro	CUG Leu	ACA Thr	AUA Ile	GGU Gly	GAG Glu	UGC Cys	CCC Pro	AAA Lys	UAU Tyr	GUA Val	AAA Lys	UCG Ser	GAG Glu	AAG Lys	1015
	310					315					320					
UUG Leu	GUC Val	UUA Leu	GCA Ala	ACA Thr	GGA Gly	CUA Leu	AGG Arg	AAU Asn	GUU Val	CCC Pro	CAG Gln	AUU Ile	GAA Glu	UCA Ser	AGA Arg	1063
325					330					335					340	
GGA Gly	UUG Leu	UUU Phe	GGG Gly	GCA Ala	AUA Ile	GCU Ala	GGU Gly	UUU Phe	AUA Ile	GAA Glu	GGA Gly	GGA Gly	UGG Trp	CAA Gln	GGA Gly	1111
				345					350					355		
AUG Met	GUU Val	GAU Asp	GGU Gly	UGG Trp	UAU Tyr	GGA Gly	UAC Tyr	CAU His	CAC His	AGC Ser	AAU Asn	GAC Asp	CAG Gln	GGA Gly	UCA Ser	1159
			360					365					370			
GGG Gly	UAU Tyr	GCA Ala	GCA Ala	GAC Asp	AAA Lys	GAA Glu	UCC Ser	ACU Thr	CAA Gln	AAG Lys	GCA Ala	UUU Phe	GAU Asp	GGA Gly	AUC Ile	1207
		375					380					385				
ACC Thr	AAC Asn	AAG Lys	GUA Val	AAU Asn	UCU Ser	GUG Val	AUU Ile	GAA Glu	AAG Lys	AUA Ile	AAC Asn	ACC Thr	CAA Gln	UUU Phe	GAA Glu	1255
	390					395					400					
GCU Ala	GUU Val	GGG Gly	AAA Lys	GAA Glu	UUC Phe	AGU Ser	AAC Asn	UUA Leu	GAG Glu	AGA Arg	AGA Arg	CUG Leu	GAG Glu	AAC Asn	UUG Leu	1303
405					410					415					420	
AAC Asn	AAA Lys	AAG Lys	AUG Met	GAA Glu	GAC Asp	GGG Gly	UUU Phe	CUA Leu	GAU Asp	GUG Val	UGG Trp	ACA Thr	UAC Tyr	AAU Asn	GCU Ala	1351
				425					430					435		
GAG Glu	CUU Leu	CUA Leu	GUU Val	CUG Leu	AUG Met	GAA Glu	AAU Asn	GAG Glu	AGG Arg	ACA Thr	CUU Leu	GAC Asp	UUU Phe	CAU His	GAU Asp	1399
			440					445					450			
UCU Ser	AAU Asn	GUC Val	AAG Lys	AAU Asn	CUG Leu	UAU Tyr	GAU Asp	AAA Lys	GUC Val	AGA Arg	AUG Met	CAG Gln	CUG Leu	AGG Arg	GAC Asp	1447
		455					460					465				
AAC Asn	GUC Val	AAA Lys	GAA Glu	CUA Leu	GGA Gly	AAU Asn	GGA Gly	UGU Cys	UUU Phe	GAA Glu	UUU Phe	UAU Tyr	CAC His	AAA Lys	UGU Cys	1495
	470					475				480						
GAU Asp	GAU Asp	GAA Glu	UGC Cys	AUG Met	AAU Asn	AGU Ser	GUG Val	AAA Lys	AAC Asn	GGG Gly	ACA Thr	UAU Tyr	GAU Asp	UAU Tyr	CCC Pro	1543
485					490					495					500	
AAG Lys	UAU Tyr	GAA Glu	GAA Glu	GAG Glu	UCU Ser	AAA Lys	CUA Leu	AAU Asn	AGA Arg	AAU Asn	GAA Glu	AUU Ile	AAA Lys	GGG Gly	GUA Val	1591
				505					510					515		
AAA Lys	UUG Leu	AGC Ser	AGC Ser	AUG Met	GGG Gly	GUU Val	UGU Cys	CGG Arg	AUC Ile	CUU Leu	GCC Ala	AUU Ile	UAU Tyr	GCU Ala	ACA Thr	1639
			520					525					530			



GUA GCA GGU UCU CUG UCA CUG GCA AUC AUG AUG GCU GGG AUC UCU UUC	1687
Val Ala Gly Ser Leu Ser Leu Ala Ile Met Met Ala Gly Ile Ser Phe	
535 540 545	
UGG AUG UGC UCC AAC GGG UCU CUG CAG UGC AGG AUC UGC AUA	1729
Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile	
550 555 560	
UGAUUAUAAG UCAUUUUUAU AUUAAAAACA CCCUUGUUUC UACU	1773

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 562 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met	Ala	Ile	Ile	Tyr	Leu	Ile	Leu	Leu	Phe	Thr	Ala	Val	Arg	Gly	Asp
1				5					10					15	
Lys	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Glu	Thr	Val	Asp
			20					25						30	
Thr	Asn	Leu	Glu	Arg	Asn	Val	Thr	Val	Thr	His	Ala	Lys	Asp	Ile	Leu
			35				40					45			
Glu	Lys	Thr	His	Asn	Gly	Lys	Leu	Cys	Lys	Leu	Asn	Gly	Ile	Pro	Pro
	50					55					60				
Leu	Glu	Leu	Gly	Asp	Cys	Ser	Ile	Ala	Gly	Trp	Leu	Leu	Gly	Asn	Pro
65					70					75				80	
Glu	Cys	Asp	Arg	Leu	Leu	Ser	Val	Pro	Glu	Trp	Ser	Tyr	Ile	Met	Glu
				85					90					95	
Lys	Glu	Asn	Pro	Arg	Asn	Gly	Leu	Cys	Tyr	Pro	Gly	Asn	Phe	Asn	Asp
			100					105					110		
Tyr	Glu	Glu	Leu	Lys	His	Leu	Leu	Ser	Ser	Val	Lys	His	Phe	Glu	Lys
			115				120					125			
Val	Lys	Ile	Leu	Pro	Lys	Asp	Arg	Trp	Ala	Gln	His	Thr	Thr	Thr	Gly
	130					135					140				



Gly	Ser	Gln	Ala	Cys	Ala	Val	Ser	Gly	Asn	Pro	Ser	Phe	Phe	Arg	Asn
145					150				155						160
Met	Val	Trp	Leu	Thr	Glu	Glu	Gly	Ser	Asn	Tyr	Pro	Val	Ala	Lys	Gly
				165					170					175	
Ser	Tyr	Asn	Asn	Thr	Ser	Gly	Glu	Gln	Met	Leu	Ile	Ile	Trp	Gly	Val
			180					185					190		
His	His	Pro	Ile	Asp	Glu	Thr	Glu	Gln	Arg	Thr	Leu	Tyr	Gln	Asn	Val
		195					200					205			
Gly	Thr	Tyr	Val	Ser	Val	Gly	Thr	Ser	Thr	Leu	Asn	Lys	Arg	Ser	Thr
	210					215					220				
Pro	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	Asn	Gly	Leu	Gly	Ser	Arg	Met
225					230					235					240
Glu	Phe	Ser	Trp	Thr	Leu	Leu	Asp	Met	Trp	Asp	Thr	Ile	Asn	Phe	Glu
				245					250					255	
Ser	Thr	Gly	Asn	Leu	Ile	Ala	Pro	Glu	Tyr	Gly	Phe	Lys	Ile	Ser	Lys
			260					265					270		
Arg	Gly	Ser	Ser	Gly	Ile	Met	Lys	Thr	Glu	Gly	Thr	Leu	Glu	Asn	Cys
		275					280					285			
Glu	Thr	Lys	Cys	Gln	Thr	Pro	Leu	Gly	Ala	Ile	Asn	Thr	Thr	Leu	Pro
	290					295					300				
Phe	His	Asn	Val	His	Pro	Leu	Thr	Ile	Gly	Glu	Cys	Pro	Lys	Tyr	Val
305					310					315					320
Lys	Ser	Glu	Lys	Leu	Val	Leu	Ala	Thr	Gly	Leu	Arg	Asn	Val	Pro	Gln
				325					330					335	
Ile	Glu	Ser	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Gly
			340					345					350		
Gly	Trp	Gln	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	Tyr	His	His	Ser	Asn
		355					360					365			
Asp	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Lys	Glu	Ser	Thr	Gln	Lys	Ala
	370					375					380				
Phe	Asp	Gly	Ile	Thr	Asn	Lys	Val	Asn	Ser	Val	Ile	Glu	Lys	Ile	Asn
385					390				395						400
Thr	Gln	Phe	Glu	Ala	Val	Gly	Lys	Glu	Phe	Ser	Asn	Leu	Glu	Arg	Arg
				405					410					415	
Leu	Glu	Asn	Leu	Asn	Lys	Lys	Met	Glu	Asp	Gly	Phe	Leu	Asp	Val	Trp
			420					425					430		



Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu  
435 440 445  
Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val Arg Met  
450 455 460  
Gln Leu Arg Asp Asn Val Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe.  
465 470 475 480  
Tyr His Lys Cys Asp Asp Glu Cys Met Asn Ser Val Lys Asn Gly Thr  
485 490 495  
Tyr Asp Tyr Pro Lys Tyr Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu  
500 505 510  
Ile Lys Gly Val Lys Leu Ser Ser Met Gly Val Cys Arg Ile Leu Ala  
515 520 525  
Ile Tyr Ala Thr Val Ala Gly Ser Leu Ser Leu Ala Ile Met Met Ala  
530 535 540  
Gly Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile  
545 550 555 560  
Cys Ile

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1467 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Influenza virus
- (B) STRAIN: wild type A/Ann Arbor/6/60 (H2N2) Egg Passage 2(3)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: NA



(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(394, "c")
- (D) OTHER INFORMATION: /product= "Neuraminidase"  
/gene= "NA"  
/note= "u in ca "master" strain; c in  
wt2(3)"  
/citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: mutation
- (B) LOCATION: replace(604, "a")
- (D) OTHER INFORMATION: /product= "Neuraminidase"  
/gene= "NA"  
/note= "u in ca "master" strain; a in  
wt2(3)"  
/citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 20..1426
- (D) OTHER INFORMATION: /product= "neuraminidase"  
/gene= "NA"  
/note= "neuraminidase protein"  
/citation= ([1])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R G
- (B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) Influenza Virus
- (C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA
- (G) DATE: 1993
- (K) RELEVANT RESIDUES IN SEQ ID NO:37: FROM 1 TO 1467



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AGCAAAAGCA GGAGUGAAA	AUG AAU CCA AAU CAA AAG ACA AUA ACA AUU GGC	52
	Met Asn Pro Asn Gln Lys Thr Ile Thr Ile Gly	
	1 5 10	
UCU GUC UCU CUC ACC AUC GCA ACA GUA UGC UUC CUC AUG CAG AUU GCC	100	
Ser Val Ser Leu Thr Ile Ala Thr Val Cys Phe Leu Met Gln Ile Ala		
	15 20 25	
AUC CUG GCA ACU ACU GUG ACA UUG CAC CUU AAG CAA CAU GAG UGC GAC	148	
Ile Leu Ala Thr Thr Val Thr Leu His Leu Lys Gln His Glu Cys Asp		
	30 35 40	
UCC CCC GCG AGC AAC CAA GUA AUG CCA UGU GAA CCA AUA AUA AUA GAA	196	
Ser Pro Ala Ser Asn Gln Val Met Pro Cys Glu Pro Ile Ile Ile Glu		
	45 50 55	
AGG AAC AUA ACA GAG AUA GUG UAU UUG AAU AAC ACC ACC AUA GAG AAA	244	
Arg Asn Ile Thr Glu Ile Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys		
	60 65 70 75	
GAG AUU UGC CCC GAA GUA GUG GGA UAC AGA AAU UGG UCA AAG CCG CAA	292	
Glu Ile Cys Pro Glu Val Val Gly Tyr Arg Asn Trp Ser Lys Pro Gln		
	80 85 90	
UGU CAA AUU ACA GGA UUU GCA CCU UUU UCU AAG GAC AAU UCA AUC CGG	340	
Cys Gln Ile Thr Gly Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg		
	95 100 105	
CUU UCU GCU GGU GGG GAC AUU UGG GUG ACG AGA GAA CCU UAU GUG UCA	388	
Leu Ser Ala Gly Gly Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser		
	110 115 120	
UGC GAC CCU GGC AAG UGU UAU CAA UUU GCA CUC GGG CAG GGG ACC ACA	436	
Cys Asp Pro Gly Lys Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr		
	125 130 135	
CUA GAC AAC AAA CAU UCA AAU GGC ACA AUA CAU GAU AGA AUC CCU CAU	484	
Leu Asp Asn Lys His Ser Asn Gly Thr Ile His Asp Arg Ile Pro His		
	140 145 150 155	
CGA ACC CUA UUA AUG AAU GAG UUG GGU GUU CCA UUU CAU UUA GGA ACC	532	
Arg Thr Leu Leu Met Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr		
	160 165 170	
AAA CAA GUG UGU GCA GCA UGG UCC AGC UCA AGU UGU CAC GAU GGA AAA	580	
Lys Gln Val Cys Ala Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys		
	175 180 185	
GCA UGG UUG CAU GUU UGU GUC ACA GGG GAU GAU AGA AAU GCA ACU GCU	628	
Ala Trp Leu His Val Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala		
	190 195 200	



AGC Ser	UUC Phe	AUU Ile	UAU Tyr	GAC Asp	GGG Gly	AAG Lys	CUU Leu	GUG Val	GAC Asp	AGU Ser	AUU Ile	GGU Gly	UCA Ser	UGG Trp	UCU Ser	676
	205					210					215					
CAA Gln	AAU Asn	GUC Val	CUC Leu	AGG Arg	ACC Thr	CAG Gln	GAG Glu	UCG Ser	GAA Glu	UGC Cys	GUC Val	UGU Cys	AUC Ile	AAU Asn	GGG Gly	724
	220				225					230					235	
ACU Thr	UGC Cys	ACA Thr	GUA Val	GUA Val	AUG Met	ACU Thr	GAU Asp	GGA Gly	AGU Ser	GCA Ala	UCA Ser	GGA Gly	AGA Arg	GCU Ala	GAU Asp	772
				240					245					250		
ACU Thr	AGA Arg	AUA Ile	CUA Leu	UUC Phe	AUU Ile	AAA Lys	GAG Glu	GGG Gly	AAA Lys	AUU Ile	GUC Val	CAU His	AUU Ile	GGC Gly	CCA Pro	820
			255					260				265				
UUG Leu	UCA Ser	GGA Gly	AGU Ser	GCU Ala	CAG Gln	CAU His	GUA Val	GAG Glu	GAG Glu	UGU Cys	UCU Ser	UGU Cys	UAC Tyr	CCU Pro	CGA Arg	868
		270					275					280				
UAU Tyr	CCU Pro	GAC Asp	GUC Val	AGA Arg	UGU Cys	AUC Ile	UGC Cys	AGA Arg	GAC Asp	AAC Asn	UGG Trp	AAA Lys	GGC Gly	UCU Ser	AAU Asn	916
	285					290					295					
AGG Arg	CCC Pro	GUU Val	AUA Ile	GAC Asp	AUA Ile	AAU Asn	AUG Met	GAA Glu	GAU Asp	UAU Tyr	AGC Ser	AUU Ile	GAU Asp	UCC Ser	AGU Ser	964
	300				305					310					315	
UAU Tyr	GUG Val	UGC Cys	UCA Ser	GGG Gly	CUU Leu	GUU Val	GGC Gly	GAC Asp	ACA Thr	CCC Pro	AGG Arg	AAC Asn	GAC Asp	GAC Asp	AGC Ser	1012
				320					325					330		
UCU Ser	AGC Ser	AAU Asn	AGC Ser	AAU Asn	UGC Cys	AGG Arg	GAU Asp	CCU Pro	AAC Asn	AAU Asn	GAG Glu	AGA Arg	GGG Gly	AAU Asn	CCA Pro	1060
			335					340				345				
GGA Gly	GUG Val	AAA Lys	GGC Gly	UGG Trp	GCC Ala	UUU Phe	GAC Asp	AAU Asn	GGA Gly	GAU Asp	GAU Asp	GUA Val	UGG Trp	AUG Met	GGA Gly	1108
		350					355					360				
AGA Arg	ACA Thr	AUC Ile	AGC Ser	AAA Lys	GAU Asp	UUA Leu	CGC Arg	UCA Ser	GGU Gly	UAU Tyr	GAA Glu	ACU Thr	UUC Phe	AAA Lys	GUC Val	1156
	365					370					375					
AUU Ile	GGU Gly	GGU Gly	UGG Trp	UCC Ser	ACA Thr	CCU Pro	AAU Asn	UCC Ser	AAA Lys	UCG Ser	CAG Gln	GUC Val	AAU Asn	AGA Arg	CAG Gln	1204
	380				385				390						395	
GUC Val	AUA Ile	GUU Val	GAC Asp	AAC Asn	AAU Asn	AAU Asn	UGG Trp	UCU Ser	GGU Gly	UAC Tyr	UCU Ser	GGU Gly	AUU Ile	UUC Phe	UCU Ser	1252
				400				405					410			
GUU Val	GAG Glu	GGC Gly	AAA Lys	AGC Ser	UGC Cys	AUC Ile	AAU Asn	AGG Arg	UGC Cys	UUU Phe	UAU Tyr	GUG Val	GAG Glu	UUG Leu	AUA Ile	1300
			415					420					425			



AGG GGA AGG CCA CAG GAG ACU AGA GUA UGG UGG ACC UCA AAC AGU AUU	1348
Arg Gly Arg Pro Gln Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile	
430 435 440	
GUU GUA UUU UGU GGC ACU UCA GGU ACU UAU GGA ACA GGC UCA UGG CCU	1396
Val Val Phe Cys Gly Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro	
445 450 455	
GAU GGG GCG AAC AUC AAU UUC AUG CCU AUA UAACGUUUCG CAAUUUUAGA	1446
Asp Gly Ala Asn Ile Asn Phe Met Pro Ile	
460 465	
AAAAAACUCC UUGUUUCUAC U	1467

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 469 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met	Asn	Pro	Asn	Gln	Lys	Thr	Ile	Thr	Ile	Gly	Ser	Val	Ser	Leu	Thr
1				5					10					15	
Ile	Ala	Thr	Val	Cys	Phe	Leu	Met	Gln	Ile	Ala	Ile	Leu	Ala	Thr	Thr
			20					25					30		
Val	Thr	Leu	His	Leu	Lys	Gln	His	Glu	Cys	Asp	Ser	Pro	Ala	Ser	Asn
		35				40						45			
Gln	Val	Met	Pro	Cys	Glu	Pro	Ile	Ile	Ile	Glu	Arg	Asn	Ile	Thr	Glu
	50					55					60				
Ile	Val	Tyr	Leu	Asn	Asn	Thr	Thr	Ile	Glu	Lys	Glu	Ile	Cys	Pro	Glu
65					70					75				80	
Val	Val	Gly	Tyr	Arg	Asn	Trp	Ser	Lys	Pro	Gln	Cys	Gln	Ile	Thr	Gly
				85					90					95	
Phe	Ala	Pro	Phe	Ser	Lys	Asp	Asn	Ser	Ile	Arg	Leu	Ser	Ala	Gly	Gly
			100					105					110		
Asp	Ile	Trp	Val	Thr	Arg	Glu	Pro	Tyr	Val	Ser	Cys	Asp	Pro	Gly	Lys
		115					120					125			



Cys	Tyr	Gln	Phe	Ala	Leu	Gly	Gln	Gly	Thr	Thr	Leu	Asp	Asn	Lys	His
130						135					140				
Ser	Asn	Gly	Thr	Ile	His	Asp	Arg	Ile	Pro	His	Arg	Thr	Leu	Leu	Met
145					150					155					160
Asn	Glu	Leu	Gly	Val	Pro	Phe	His	Leu	Gly	Thr	Lys	Gln	Val	Cys	Ala
				165					170					175	
Ala	Trp	Ser	Ser	Ser	Ser	Cys	His	Asp	Gly	Lys	Ala	Trp	Leu	His	Val
			180					185					190		
Cys	Val	Thr	Gly	Asp	Asp	Arg	Asn	Ala	Thr	Ala	Ser	Phe	Ile	Tyr	Asp
		195					200					205			
Gly	Lys	Leu	Val	Asp	Ser	Ile	Gly	Ser	Trp	Ser	Gln	Asn	Val	Leu	Arg
	210					215					220				
Thr	Gln	Glu	Ser	Glu	Cys	Val	Cys	Ile	Asn	Gly	Thr	Cys	Thr	Val	Val
225					230					235					240
Met	Thr	Asp	Gly	Ser	Ala	Ser	Gly	Arg	Ala	Asp	Thr	Arg	Ile	Leu	Phe
				245					250					255	
Ile	Lys	Glu	Gly	Lys	Ile	Val	His	Ile	Gly	Pro	Leu	Ser	Gly	Ser	Ala
			260					265					270		
Gln	His	Val	Glu	Glu	Cys	Ser	Cys	Tyr	Pro	Arg	Tyr	Pro	Asp	Val	Arg
		275					280					285			
Cys	Ile	Cys	Arg	Asp	Asn	Trp	Lys	Gly	Ser	Asn	Arg	Pro	Val	Ile	Asp
	290					295					300				
Ile	Asn	Met	Glu	Asp	Tyr	Ser	Ile	Asp	Ser	Ser	Tyr	Val	Cys	Ser	Gly
305					310					315					320
Leu	Val	Gly	Asp	Thr	Pro	Arg	Asn	Asp	Asp	Ser	Ser	Ser	Asn	Ser	Asn
				325					330					335	
Cys	Arg	Asp	Pro	Asn	Asn	Glu	Arg	Gly	Asn	Pro	Gly	Val	Lys	Gly	Trp
			340					345					350		
Ala	Phe	Asp	Asn	Gly	Asp	Asp	Val	Trp	Met	Gly	Arg	Thr	Ile	Ser	Lys
		355					360					365			
Asp	Leu	Arg	Ser	Gly	Tyr	Glu	Thr	Phe	Lys	Val	Ile	Gly	Gly	Trp	Ser
	370					375					380				
Thr	Pro	Asn	Ser	Lys	Ser	Gln	Val	Asn	Arg	Gln	Val	Ile	Val	Asp	Asn
385					390					395					400
Asn	Asn	Trp	Ser	Gly	Tyr	Ser	Gly	Ile	Phe	Ser	Val	Glu	Gly	Lys	Ser
				405					410					415	



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Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Gln  
                  420                                  425                                  430  
Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly  
                  435                                  440                                  445  
Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile  
                  450                                  455                                  460  
Asn Phe Met Pro Ile  
465

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1566 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Influenza virus

(B) STRAIN: wild type A/Ann Arbor/6/60 (H2N2) Egg Passage 2(3)

(vii) IMMEDIATE SOURCE:

(B) CLONE: NP

(ix) FEATURE:

(A) NAME/KEY: mutation

(B) LOCATION: replace(113, "a")

(D) OTHER INFORMATION: /note= "c in ca "master" strain; a in  
wt2(3); c in 1988 reported wild type  
E28-32 strain (manuscript) but a in 1988  
reported wild type E28-32 strain  
/citation= ([1][2])



(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(146, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(627, "c")

(D) OTHER INFORMATION: /note= "c in ca "master" strain and in  
wt2(3); a in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(909, "g")

(D) OTHER INFORMATION: /note= "g in ca "master" strain and in  
wt2(3); c in 1988 reported wild type  
E28-32 strain"  
/citation= ([1][2])

(ix) FEATURE:

(A) NAME/KEY: conflict

(B) LOCATION: replace(1550, "a")

(D) OTHER INFORMATION: /note= "a in ca "master" strain and in  
wt2(3); deletion in 1988 reported wild  
type E28-32 strain"  
/citation= ([1][2])



(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 46..1539
- (D) OTHER INFORMATION: /product= "Nucleoprotein"  
/gene= "NP"  
/note= "nucleoprotein"  
/citation= ([1][2])

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Herlocher, M L  
Maassab, H F  
Webster, R W
- (B) TITLE: Molecular and biological changes in the cold adapted  
master strain A/AA/6/60 (H2N2) influenza virus
- (C) JOURNAL: Proceedings of the National Academy of Sciences of  
the USA
- (G) DATE: 1993
- (K) RELEVANT RESIDUES IN SEQ ID NO:39: FROM 1 TO 1566

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Cox, N J  
Kitame, F  
Kendal, A P  
Maassab, H F  
Naeve, C
- (B) TITLE: Identification of sequence changes in the cold-adapted  
live attenuated influenza vaccine strain, A/Ann  
Arbor/6/60 (H2N2)
- (C) JOURNAL: Virology
- (D) VOLUME: 167
- (F) PAGES: 554-567
- (G) DATE: 1988
- (K) RELEVANT RESIDUES IN SEQ ID NO:39: FROM 1 TO 1566



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AGCAAAAGCA GGGUAGAUAA UCACUCACUG AGUGACAUCA AAAUC AUG GCG UCC	54
Met Ala Ser	
1	
CAA GGC ACC AAA CGG UCU UAU GAA CAG AUG GAA ACU GAU GGG GAA CGC	102
Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp Gly Glu Arg	
5 10 15	
CAG AAU GCA AAU GAA AUC AGA GCA UCC GUC GGG AAG AUG AUU GGU GGA	150
Gln Asn Ala Asn Glu Ile Arg Ala Ser Val Gly Lys Met Ile Gly Gly	
20 25 30 35	
AUU GGA CGA UUC UAC AUC CAA AUG UGC ACC GAA CUU AAA CUC AGU GAU	198
Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys Leu Ser Asp	
40 45 50	
UAU GAG GGG CGG CUG AUC CAG AAC AGC UUA ACA AUA GAG AGA AUG GUG	246
Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu Arg Met Val	
55 60 65	
CUC UCU GCU UUU GAC GAG AGG AGG AAU AAA UAU CUG GAA GAA CAU CCC	294
Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu Glu His Pro	
70 75 80	
AGC GCG GGG AAG GAU CCU AAG AAA ACU GGA GGA CCC AUA UAC AAG AGA	342
Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile Tyr Lys Arg	
85 90 95	
GUA GAU GGA AAG UGG AUG AGG GAA CUC GUC CUU UAU GAC AAA GAA GAA	390
Val Asp Gly Lys Trp Met Arg Glu Leu Val Leu Tyr Asp Lys Glu Glu	
100 105 110 115	
AUA AGG CGA AUC UGG CGC CAA GCU AAU AAU GGU GAU GAU GCA ACA GCU	438
Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Asp Asp Ala Thr Ala	
120 125 130	
GGU CUG ACU CAC AUG AUG AUC UGG CAU UCC AAU UUG AAU GAU ACA ACA	486
Gly Leu Thr His Met Met Ile Trp His Ser Asn Leu Asn Asp Thr Thr	
135 140 145	
UAC CAG AGG ACA AGA GCU CUU GUU CGC ACC GGA AUG GAU CCC AGG AUG	534
Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp Pro Arg Met	
150 155 160	
UGC UCU UUG AUG CAG GGU UCG ACU CUC CCU AGG AGG UCU GGA GCC GCA	582
Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser Gly Ala Ala	
165 170 175	
GGC GCU GCA GUC AAA GGA GUU GGG ACA AUG GUG AUG GAG UUG AUC AGG	630
Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Met Glu Leu Ile Arg	
180 185 190 195	



AUG Met	AUC Ile	AAA Lys	CGU Arg	GGG Gly 200	AUC Ile	AAU Asn	GAU Asp	CGG Arg	AAC Asn 205	UUC Phe	UGG Trp	AGA Arg	GGU Gly	GAG Glu 210	AAU Asn	678
GGG Gly	CGG Arg	AAA Lys	ACA Thr 215	AGG Arg	AAU Asn	GCU Ala	UAU Tyr	GAG Glu 220	AGA Arg	AUG Met	UGC Cys	AAC Asn	AUU Ile 225	CUC Leu	AAA Lys	726
GGA Gly	AAA Lys	UUU Phe 230	CAA Gln	ACA Thr	GCU Ala	GCA Ala	CAA Gln 235	AGA Arg	GCA Ala	AUG Met	AUG Met	GAU Asp 240	CAA Gln	GUG Val	AGA Arg	774
GAA Glu 245	AGC Ser	CGG Arg	AAC Asn	CCA Pro	GGA Gly	AAU Asn 250	GCU Ala	GAG Glu	AUC Ile	GAA Glu	GAU Asp 255	CUC Leu	AUG Ile	UUU Phe	CUG Leu	822
GCA Ala 260	CGG Arg	UCU Ser	GCA Ala	CUC Leu	AUA Ile 265	UUG Leu	AGA Arg	GGG Gly	UCA Ser	GUU Val 270	GCU Ala	CAC His	AAA Lys	UCU Ser	UGU Cys 275	870
CUG Leu	CCU Pro	GCC Ala	UGU Cys	GUG Val 280	UAU Tyr	GGA Gly	CCU Pro	GCC Ala	GUA Val 285	GCC Ala	AGU Ser	GGG Gly	UAC Tyr	GAC Asp 290	UUC Phe	918
GAA Glu	AAA Lys	GAG Glu	GGA Gly 295	UAC Tyr	UCU Ser	UUA Leu	GUA Val	GGG Gly 300	AUA Ile	GAC Asp	CCU Pro	UUC Phe	AAA Lys 305	CUG Leu	CUU Leu	966
CAA Gln	AAC Asn	AGC Ser 310	CAA Gln	GUA Val	UAC Tyr	AGC Ser	CUA Leu 315	AUC Ile	AGA Arg	CCG Pro	AAU Asn	GAG Glu 320	AAU Asn	CCA Pro	GCA Ala	1014
CAC His 325	AAG Lys	AGU Ser	CAG Gln	CUG Leu	GUG Val 330	UGG Trp	AUG Met	GCA Ala	UGC Cys	AAU Asn	UCU Ser 335	GCU Ala	GCA Ala	UUU Phe	GAA Glu	1062
GAU Asp 340	CUA Leu	AGA Arg	GUA Val	UCA Ser	AGC Ser 345	UUC Phe	AUC Ile	AGA Arg	GGG Gly	ACC Thr 350	AAA Lys	GUA Val	AUC Ile	CCA Pro	AGG Arg 355	1110
GGG Gly	AAA Lys	CUU Leu	UCC Ser	ACU Thr 360	AGA Arg	GGA Gly	GUA Val	CAA Gln	AUU Ile 365	GCU Ala	UCA Ser	AAU Asn	GAA Glu	AAC Asn	AUG Met 370	1158
GAU Asp	ACU Thr	AUG Met	GGA Gly 375	UCA Ser	AGU Ser	ACU Thr	CUU Leu	GAA Glu 380	CUG Leu	AGA Arg	AGC Ser	AGG Arg	UAC Tyr 385	UGG Trp	GCC Ala	1206
AUA Ile	AGG Arg	ACC Thr 390	AGA Arg	AGU Ser	GGA Gly	GGA Gly	AAC Asn 395	ACU Thr	AAU Asn	CAA Gln	CAG Gln	AGG Arg 400	GCC Ala	UCU Ser	GCA Ala	1254
GGU Gly 405	CAA Gln	AUC Ile	AGU Ser	GUA Val	CAA Gln	CCU Pro 410	ACG Thr	UUU Phe	UCU Ser	GUG Val	CAA Gln 415	AGA Arg	AAC Asn	CUC Leu	CCA Pro	1302



UUU GAC AAA CCA ACC AUC AUG GCA GCA UUC ACU GGG AAU GCA GAG GGA Phe Asp Lys Pro Thr Ile Met Ala Ala Phe Thr Gly Asn Ala Glu Gly 420 425 430 435	1350
AGA ACA UCA GAC AUG AGG GCA GAA AUC AUA AGG AUG AUG GAA GGU GCA Arg Thr Ser Asp Met Arg Ala Glu Ile Ile Arg Met Met Glu Gly Ala 440 445 450	1398
AAA CCA GAA GAA GUG UCC UUC CAG GGG CGG GGA GUC UUC GAG CUC UCG Lys Pro Glu Glu Val Ser Phe Gln Gly Arg Gly Val Phe Glu Leu Ser 455 460 465	1446
GAC GAA AAG GCA ACG AAC CCG AUC GUG CCC UCU UUU GAC AUG AGU AAU Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp Met Ser Asn 470 475 480	1494
GAA GGA UCU UAU UUC UUC GGA GAC AAU GCA GAG GAG UAC GAC AAU Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr Asp Asn 485 490 495	1539
UAAGGAAAAA AUACCCUUGU UUCUACU	1566

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 498 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Ala Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp 1 5 10 15
Gly Glu Arg Gln Asn Ala Asn Glu Ile Arg Ala Ser Val Gly Lys Met 20 25 30
Ile Gly Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys 35 40 45
Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu 50 55 60
Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu 65 70 75 80



Glu	His	Pro	Ser	Ala	Gly	Lys	Asp	Pro	Lys	Lys	Thr	Gly	Gly	Pro	Ile
				85					90					95	
Tyr	Lys	Arg	Val	Asp	Gly	Lys	Trp	Met	Arg	Glu	Leu	Val	Leu	Tyr	Asp
			100					105					110		
Lys	Glu	Glu	Ile	Arg	Arg	Ile	Trp	Arg	Gln	Ala	Asn	Asn	Gly	Asp	Asp
		115					120					125			
Ala	Thr	Ala	Gly	Leu	Thr	His	Met	Met	Ile	Trp	His	Ser	Asn	Leu	Asn
	130					135					140				
Asp	Thr	Thr	Tyr	Gln	Arg	Thr	Arg	Ala	Leu	Val	Arg	Thr	Gly	Met	Asp
145					150					155					160
Pro	Arg	Met	Cys	Ser	Leu	Met	Gln	Gly	Ser	Thr	Leu	Pro	Arg	Arg	Ser
				165					170					175	
Gly	Ala	Ala	Gly	Ala	Ala	Val	Lys	Gly	Val	Gly	Thr	Met	Val	Met	Glu
			180					185					190		
Leu	Ile	Arg	Met	Ile	Lys	Arg	Gly	Ile	Asn	Asp	Arg	Asn	Phe	Trp	Arg
		195					200					205			
Gly	Glu	Asn	Gly	Arg	Lys	Thr	Arg	Asn	Ala	Tyr	Glu	Arg	Met	Cys	Asn
	210					215					220				
Ile	Leu	Lys	Gly	Lys	Phe	Gln	Thr	Ala	Ala	Gln	Arg	Ala	Met	Met	Asp
225					230					235					240
Gln	Val	Arg	Glu	Ser	Arg	Asn	Pro	Gly	Asn	Ala	Glu	Ile	Glu	Asp	Leu
				245					250					255	
Ile	Phe	Leu	Ala	Arg	Ser	Ala	Leu	Ile	Leu	Arg	Gly	Ser	Val	Ala	His
			260					265					270		
Lys	Ser	Cys	Leu	Pro	Ala	Cys	Val	Tyr	Gly	Pro	Ala	Val	Ala	Ser	Gly
		275					280					285			
Tyr	Asp	Phe	Glu	Lys	Glu	Gly	Tyr	Ser	Leu	Val	Gly	Ile	Asp	Pro	Phe
	290					295					300				
Lys	Leu	Leu	Gln	Asn	Ser	Gln	Val	Tyr	Ser	Leu	Ile	Arg	Pro	Asn	Glu
305					310						315				320
Asn	Pro	Ala	His	Lys	Ser	Gln	Leu	Val	Trp	Met	Ala	Cys	Asn	Ser	Ala
				325					330					335	
Ala	Phe	Glu	Asp	Leu	Arg	Val	Ser	Ser	Phe	Ile	Arg	Gly	Thr	Lys	Val
			340					345					350		
Ile	Pro	Arg	Gly	Lys	Leu	Ser	Thr	Arg	Gly	Val	Gln	Ile	Ala	Ser	Asn
		355					360					365			



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Glu Asn Met Asp Thr Met Gly Ser Ser Thr Leu Glu Leu Arg Ser Arg  
370 375 380

Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg  
385 390 395 400

Ala Ser Ala Gly Gln Ile Ser Val Gln Pro Thr Phe Ser Val Gln Arg  
405 410 415

Asn Leu Pro Phe Asp Lys Pro Thr Ile Met Ala Ala Phe Thr Gly Asn  
420 425 430

Ala Glu Gly Arg Thr Ser Asp Met Arg Ala Glu Ile Ile Arg Met Met  
435 440 445

Glu Gly Ala Lys Pro Glu Glu Val Ser Phe Gln Gly Arg Gly Val Phe  
450 455 460

Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp  
465 470 475 480

Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr  
485 490 495

Asp Asn